American Journal of Diseases of Children

VOLUME 70 SEPTEMBER 1945 NUMBER 3

COPYRIGHT, 1945, BY THE AMERICAN MEDICAL ASSOCIATION

# HUMAN MILK STUDIES

## XIX. IMPLICATIONS OF BREAST FEEDING AND THEIR INVESTIGATION

ICIE G. MACY, PH.D.; HAROLD H. WILLIAMS, PH.D.
J. P. PRATT, M.D., AND BRENTON M. HAMIL, M.D.
DETROIT

The kind and the amount of food consumed by people during infancy, childhood and the reproductive period predetermine in a large measure the future health and strength of the nation. Infants, born and yet to be born, are the citizens of the future, and the foundation of the physical fitness of a people is embedded in the feeding and the care of the nation's infants. Food must be adequate in amount and carry all the essential materials for the formation, the repair and the regulation of tissue during the formative periods—gestation, infancy and childhood—if the health foundation under the nation is to be stable, efficient and invincible. Conditioning factors coincident with pregnancy, parturition and lactation may increase the food requirements of the mother, as growth and development do those of the child, hence provision must be made to take care of these newly acquired and changing needs.[1]

The milk of a healthy mother has long been acknowledged to be the "most nearly perfect food" for the infant.[2] If her food is sufficient in amount and in its content of nutriments, and her body is well nourished at the time of conception, she has an excellent chance of producing a strong and healthy infant—she herself pursuing a healthful and fruitful existence both while carrying the child and while feeding it by breast. Reports assembled during the war years show that maternal mortality is on the decline—this in spite of the conditions in wartime, when so many physicians are serving with the armed forces and all hospitals have terrific shortages of personnel. For this saving of mothers' lives credit[3] has been given to several important factors, namely: (*a*) a better understanding by the public of the need for good medical and hospital care; (*b*) the fact that more women are now seeing their physicians early in pregnancy and receiving medical supervision until after the baby is borne; (*c*) the federal programs which finance medical nursing and hospital care for wives of men in the four lowest pay grades of the armed services. One important fact the report failed to recognize: that in this country, although in times of crises food shortages usually occur,[4] economic conditions have improved and more and better food is now available to a larger portion of the population. The availability of food, the improved methods of growing and handling foods to enhance and preserve their maximum nutritive values and the worldwide campaign for better nutrition are now being reflected in better health, and mothers have become better obstetric risks; their babies are stronger and nutritionally more stable.[5]

On the average, the breast-fed infant enjoys a better chance to live and to have fewer ill-

From the Research Laboratory, Children's Fund of Michigan (I. G. Macy and H. H. Williams) and the Henry Ford Hospital (Drs. J. P. Pratt and B. M. Hamil).

1. Jolliffe, N.: Conditioned Malnutrition, in Handbook of Nutrition, Chicago, American Medical Association Press, 1943, pp. 521-543.

2. (*a*) Ebbs, J. H.: Nutritive Requirements in Pregnancy and Lactation, in Handbook of Nutrition, Chicago, American Medical Association Press, 1943, pp. 385-402. (*b*) Jeans, P. C.: The Feeding of Healthy Infants and Children, ibid., pp. 339-363.

3. Michigan Maternal Deaths Were Under 200 Last Year for First Time in History, editorial, Michigan Pub. Health **33**:83-85 (May) 1945.

4. Cannon, P. R.: War, Famine and Pestilence, Scient. Monthly **56**:5-14 (Jan.) 1943.

5. Hummel, F. C.; Hunscher, H. A.; Bates, M. F.; Bonner, P.; Macy, I. G., and Johnston, J. A.: A Consideration of the Nutritive State in the Metabolism of Women During Pregnancy, J. Nutrition **13**:263-278 (March) 1937. Ebbs, J. H.; Scott, W. A.; Tisdall, F. F.; Moyle, W. J., and Bell, M.: Nutrition in Pregnancy, Canad. M. A. J. **46**:1-6 (Jan.) 1942. Burke, B. S.; Beal, V. A.; Kirkwood, S. B., and Stuart, H. C.: The Influence of Nutrition During Pregnancy upon the Condition of the Infant at Birth, J. Nutrition **26**:569-583 (Dec.) 1943. Bethell, F. H.; Blecha, E., and Van Sant, J. H.: Nutritional Inadequacies in Pregnancy Correlated with the Incidence of Anemia, J. Am. Dietet. A. **19**:165-172 (March) 1943. Balfour, M. I.: Supplementary Feeding in Pregnancy, Lancet **1**:208-211 (Feb. 12) 1944. Sontag, L. W.; Reynolds, E. L., and Torbet, V.: Status of Infant at Birth as Related to Basal Metabolism of Mother in Pregnancy, Am. J. Obst. & Gynec. **48**:208-214 (Aug.) 1944.

nesses.[6] Breast feeding, therefore, has new significance when medical care is limited by the reduced number of physicians and nurses available. To the parents, breast feeding represents tangible savings of time, labor and money, in addition to the intangible values which are far more important, even if less easily assessed. The obstetrician (J. P. P.) who collaborated in the investigation of human milk for which this paper is the introductory report, has said:

Selection, encouragement and following through a suitable group of mothers for the cooperative milk study has been my pleasant duty—"pleasant" because the subject has been intensely interesting, "duty" because it is fundamental to the welfare of mankind.

Coming down through the ages, it has been natural for a mother to nurse her child. However, with the advancement of civilization and science, substitutes have been found for mother's milk which are seemingly adequate. Hence, the mother who lacks maternal instinct or finds it conducive to her pleasure and time accepts these substitutes without a qualm as to their inferiority or as to the damage to the health and future welfare of her child. A campaign to reeducate is timely. Such is the purpose of this project.

Not enough stress has been placed on the emotional relationship between the nursing mother and the child. Not only does it make the bond between them stronger (as witness the former wetnurse and her charge) but even in the early months of the child's life gives a pronounced sense of security to it. Just as a woman who has never had a child feels that she is being thwarted, so a woman who does not nurse a child is being thwarted of a natural bond. Her physical obligation to a child begins at the onset of pregnancy and carries through the lactation period. It is not enough to bring the child into the world and then feel that all obligation for his health and well-being has been fulfilled.

There is a definite sense of loss if a woman does not nurse her child. Even after years she may be heard to remark, "I wish I could have nursed my baby." With encouragement and study of their particular problem 85 per cent of women could have the supreme satisfaction of knowing that they have completed their physical duty. It is not only scientists, but painters and writers, who have glorified the act of nursing a baby. It gives a woman a sense of possessiveness, satisfaction and pleasure that nothing else can equal. She is nourishing the child from her body, giving it life, more so than at the moment of birth.

In this research, the various values to be found in mother's milk have been established. This knowledge coupled with a normal woman's native desire to nurse her child, if properly presented to a prospective mother, should induce her to do the natural thing—nurse her child.

6. Grulee, C. G.; Sanford, H. N., and Herron, P. H.: Breast and Artificial Feeding: Influence on Morbidity and Mortality of Twenty Thousand Infants, J. A. M. A. **103**:735-738 (Sept. 8) 1934. Grulee, C. G.; Sanford, H. N., and Schwartz, H.: Breast and Artificially Fed Infants: A Study of the Age Incidence in the Morbidity and Mortality in Twenty Thousand Cases, ibid. **104**:1986-1988 (June 1) 1935.

After consecutive observations of 900 women the same author (J. P. P.)[7] stated:

. . . In general our results show that if the mother attempts breast nursing the chances are approximately 87 out of 100 that on the fifth postpartum day she will have an adequate milk supply to continue nursing. The chances for full breast nursing are from 65 to 7 per cent, depending upon the age of the patient, parity and whether the baby is nursed from alternate or both breasts at each feeding.

There is a deficiency of lactation in approximately per cent of mothers attempting to nurse their babies. . . . Many cases will increase to part time nursing without treatment. No case of deficient lactation in this study of 900 consecutive cases increased to full breast nursing by the tenth postpartum day.

Within the same Detroit environs, hospital reports indicate that breast feeding varies from 5 to 69 per cent.[8] What is the cause for so great a variation in the number of women who feed their babies by breast? The responsibility must be shared by mothers with the physicians and the nurses under whose care they place themselves.

It is well known that mothers shop for a doctor who will promise a painless birth or contrariwise, for one who uses anesthetic and analgesic drugs only in emergencies, and then sparingly; for a doctor who is not interested in breast feeding and who recommends artificial formulas or, in contrast, for one who believes wholeheartedly that both mother and infant profit physically and emotionally through breast feeding. If the doctor is a proponent of breast feeding, he will usually, from the initial visit, prepare the mother mentally and physically for feeding her child by breast. Moreover, he will train his nurses and technicians to support his ideas. On the other hand, the doctor who is interested primarily in the mechanics of labor and parturition and in the immediate welfare of the mother and the infant during this period and is indifferent to the future welfare of the baby, is usually surrounded by a nursing staff which has no interest and little time for initiating the mother and the infant into the technic and procedure of successful breast feeding and which gives no encouragement to the mother when difficulties are to be overcome.

In the study referred to[7] it was found with respect to the babies who were never put to the breast that:

7. Stewart, H. L., and Pratt, J. P.: Influence of Suckling Stimulus on Lactation, West. J. Surg. 98-103 (Feb.) 1941.

8. Byington, G. M., and Harmon, G. E.: In the Interest of Infant Health, Detroit M. News (March 27) 1944.

. . . The most important single factor in this group was refusal on the part of the mother to attempt nursing. This accounted for 40 per cent of the mothers not nursing their babies. Undoubtedly this group would have been much greater had many others not been urged to breast feed their babies. The most conspicuous reasons for mothers not attempting to nurse included: lack of maternal interest; fear of pendulous breasts and unattractive physical appearance; business, home, or social duties; and belief that bottle feeding is equal to or even superior to breast nursing. Many multiparae stated that previous breast nursing had been discontinued because the milk was "too thin" or "too rich" and "did not agree with the baby." We encountered no instance [i. e., in 900 cases] of so-called "abnormal milk." These terms are erroneously applied as indicating some qualitative changes in the breast milk, whereas the problem usually involves question of the quantity of milk ingested by the baby. In this study it was not necessary to remove any baby from breast because the milk did not agree with it.

There is a definite individual physiologic capacity of the mammary gland, shown both in the total quantity of milk which can be produced and in the length of time that lactation persists under normal conditions. Within limits the quantity of milk secreted by a normal breast adjusts itself to the demand.[9]

Reference to the clinical effect of the suckling stimulus on lactation frequently is made, but with few supporting facts. Data have been presented[10] which suggest that the suckling stimulus may account for the higher incidence of full breast nursing among primiparas who place their babies early on both breasts, a suggestion which is supported by others[11] who have demonstrated the important role of the suckling stimulus in the lactation of normal animals. In observations on women[7] it was observed that

. . . the suckling stimulus does not in any way increase milk production in the presence of a part time or deficient state. Apparently this suckling stimulus plays an important part in the development of mammary gland activity among primiparae during the first lactation . . . once lactation is established, an added suckling stimulus in the human plays no significant part in increasing deficient or part time nursing. . . . If they [the breasts] are capable of greater secretion under additional demands, milk production increases.

Increase in output can be accomplished by placing another infant on the breasts or by completely emptying the breasts by other means at frequent intervals. The stimulation of an increased flow by completing expression of the milk after the baby has nursed can be demonstrated soon after delivery and throughout the whole lactation period. It has been demonstrated that lactation may persist at a high level over fourteen months or more in the total absence of the suckling reflex, and some women with inverted nipples and with a persistent desire to raise their babies on breast milk have successfully expressed their milk manually and fed the milk by bottle.[9]

At times not only do the most carefully prepared scientific formulas fail to satisfy the nutritive needs of a particular infant, but even mother's milk may fail to do so. With better understanding of the newer nutritional principles and recognition that a mother's milk may not always spell nutritive success for an infant it has become necessary to revaluate the dietary properties of Nature's food—mother's milk—and to reconsider some of the principles on which the feeding of infants hitherto has been based. The present goals in the feeding of infants are the stimulation of wider use of breast feeding and human milk and the application of nutritional principles to the mother's diet so that she may produce breast milk that is adequate both in quantity and in quality. When breast feeding is contraindicated, the ultimate goal is to provide a formula which is superior to human milk for the infants.

The milk secreted by different women varies in composition between wide limits.[12] It has been demonstrated that the quality and the

9. Macy, I. G.; Hunscher, H. A.; Donelson, E., and Nims, B.: Human Milk Flow, Am. J. Dis. Child. **39**: 1186-1204 (June) 1930.

10. Stewart, H. L., and Pratt, J. P.: Effect of Prolactin on Mammary Gland Secretion, Endocrinology **25**: 347-353 (Sept.) 1939.

11. Selye, H.: On the Nervous Control of Lactation, Am. J. Physiol. **107**:535-538 (March) 1934. Selye, H.; Collip, J. B., and Thomson, D. L.: Nervous and Hormone Factors in Lactation, Endocrinology **18**:237-248 (March) 1934. Reece, R. P., and Turner, C. W.: Influence of Suckling upon Galactin Content of Rat Pituitary, Proc. Soc. Exper. Biol. & Med. **35**:367-368 (Dec.) 1936; Effect of Stimulus of Suckling upon Galactin Content of the Rat Pituitary, ibid. **35**:621-622 (Jan.) 1937.

12. Nims, B.; Macy, I. G.; Brown, M., and Hunscher, H. A.: Human Milk Studies: IX. Variations in the Composition of Milk at Four Hour Intervals During the Day and Night, Am. J. Dis. Child. **43**:828-844 (April) 1932. Macy, I. G.; Nims, B.; Brown, M., and Hunscher, H. A.: Human Milk Studies: VII. Chemical Analysis of Milk Representative of the Entire First and Last Halves of the Nursing Period, ibid. **42**:569-589 (Sept.) 1931. Brown, M.; Macy, I. G.; Nims, B., and Hunscher, H. A.: Human Milk Studies: VIII. A Comparison of the Composition of the Milk from the Two Breasts, ibid. **43**:40-51 (Jan.) 1932. Nims, B.; Macy, I. G.; Hunscher, H. A., and Brown, M.: Human Milk Studies: X. Daily and Monthly Variations in Milk Components as Observed in Two Successive Lactation Periods, ibid. **43**: 1062-1076 (May) 1932.

quantity of milk secreted may be altered by the following conditions: amount and kind of food consumed; environmental conditions, such as fresh air, sunshine, amount and intensity of work, rest and exercise; disease; size and anatomic structure of the mammary gland; heredity, and the emotional makeup of the subject.

In quantitative studies of the dietaries of mothers [13] with respect to the nutriments secreted into breast milk, it has been observed that some constituents are interconvertible in metabolism. The fat and carbohydrates of milk are not necessarily traceable directly to the corresponding food substances. Protein may be transformed into dextrose, and dextrose into fat. Proteins, fats and carbohydrates have been shown to serve as the initial sources of milk fat. However, the mammary glands must compete with other organs for nutriments from the blood stream during the metabolic processes; consequently there is a variation in the quantities and the proportions of the various components available at any given time for the synthesis of milk. This variation is influenced by digestion, absorption and environmental factors.

When the necessary ingredients for the synthesis of milk are lacking in the daily food, the mother's output of milk may be decreased or the milk may become deficient in these constituents, or the needed elements may be drawn from her body tissues. A marked deficiency of dietary proteins, fats or carbohydrates is usually associated with a reduction in milk flow [14]; an inadequate intake of vitamins may result in a milk deficient in vitamins,[15] while an insufficient intake of minerals may cause a drain on the maternal reserve.[16] The final test of any food for an infant, whether it be breast milk or modified cow's milk, is the type of growth it produces and whether it produces a nutritionally stable, healthy infant who is resistant to infections and unhampered in physical and mental growth and development. Therefore, a complete study of the milk of mothers should include detailed observations on the physical status of the infants. The healthiest baby may not necessarily be the one that grows the fastest or the one that has the largest bones and lays down the most calcium in a given period.

To determine a standard for the composition of breast milk it is necessary to observe the full flow of milk produced by healthy mothers who are ingesting diets of known composition—diets that maintain the mothers in good health and enable them to supply milk containing the nutriments essential for the satisfactory growth of their babies. These observations can well be supplemented by determining the composition of milk produced by a number of women while they are successfully breast-feeding their infants. From such data it is possible to determine with accuracy the average amounts of the nutriments incorporated in breast milk which represent the quantities Nature thought it necessary to provide. With a similar study on cow's milk [17] it is possible to make direct comparisons of the nutriments contained in the two milks that are generally used in infant feeding. With knowledge of the standard composition of the two milks, modifications of cow's milk may be made with greater accuracy and, consequently, greater security.

It has been demonstrated that cow's milk, having a higher concentration of minerals, may permit a larger retention of minerals over the few months of infancy. No one knows whether the biggest baby, or the one who stores the most calcium, has a greater advantage throughout life than the infant fed breast milk, who may grow more slowly but with greater nutritional stability and may extend that steady growth farther into childhood. Indeed, studies of

13. Shukers, C. F.; Macy, I. G.; Donelson, E.; Nims, B., and Hunscher, H. A.: Sources of Nutrients Chosen by Women During Pregnancy, Lactation and Reproductive Rest, J. Am. Dietet. A. **7**:235-251 (Dec.) 1931. Shukers, C. F.; Macy, I. G.; Nims, B.; Donelson, E., and Hunscher, H. A.: A Quantitative Study of the Dietary of the Human Mother with Respect to the Nutrients Secreted into Breast Milk, J. Nutrition **5**:127-139 (March) 1932.

14. Garry, R. C., and Stiven, D.: A Review of Recent Work on Dietary Requirements in Pregnancy and Lactation, with an Attempt to Assess Human Requirements, Nutrition Abstr. & Rev. **5**:855-887 (April) 1936. Ebbs, J. H.; Tisdall, F. F., and Scott, W. A.: The Influence of Prenatal Diet on the Mother and Child, J. Nutrition **22**:515-526 (Nov.) 1941. Ebbs.[2a] Jeans.[2b]

15. Andrews, V. L.: Infantile Beriberi, Philippine J. Sc., Sect. B **7**:67 (April) 1912.

16. Hunscher, H. A.: Metabolism of Women During the Reproductive Cycle: II. Calcium and Phosphorus Utilization in Two Successive Lactation Periods, J. Biol. Chem. **86**:37-57 (March) 1930. Donelson, E.; Nims, B.; Hunscher, H. A., and Macy, I. G.: Metabolism of Women During the Reproductive Cycle: IV. Calcium and Phosphorus Utilization in Late Lactation and During Subsequent Reproductive Rest, ibid. **91**:675-686 (May) 1931.

17. A paper which is a part of the work on cow's milk by the Cornell group is included in this series: Lawrence, J. M.; Herrington, B. L., and Maynard, L. A.: Human Milk Studies: XXVII. Comparative Values of Bovine and Human Milk in Infant Feeding, Am. J. Dis. Child. this issue, p. 193.

animals indicate that years may be added to the life span and life to the years by giving diets complete in all known dietary essentials which produce slow but steady growth. Certain of the essential amino acids appear in different proportions in the two milks,[18] and the proportions of the vitamins in the two milks may be different. May not the fact that the infant and the calf have different types of digestive apparatus and are endowed with different rates of growth and development have some bearing on the kind of food which Nature has provided for each of them? Perhaps the type of growth inaugurated by good breast milk may be controlled by the vitamin, protein and mineral mixture best suited to the infant. There is need for a revaluation of both human and bovine milk with respect to all the known nutriments, and this and subsequent series of studies were planned to fill this need in part.

The success of any investigation with human subjects is dependent on the selection of suitable participants and the maintenance of such control that similar procedures will produce data that are comparable. In studying lactation and the composition of human milk the cooperation of the women is of prime importance. Personality and emotional factors must be considered with medical history and physical status, for the interest of some prospective mothers in joining in a contribution to science would wane during the discomfitures of pregnancy or be diminished by family reaction, or would fail to meet the rigors of experimental routine superimposed on the experience of childbirth and the demands of the newborn infant.

To obtain valid data on the normal composition of human milk, the environment must be favorable to close control throughout pregnancy and lactation without disturbance of the course of events during the reproductive cycle. Provision must be made to sample the full twenty-four hour production of milk under conditions approximating the nursing regimen, with maintenance of aseptic conditions and without disadvantage to mother or child. The regimen must be consistent for all subjects, and diets must be comparable qualitatively if the data are to be combined. To these requirements must be added that of a competent laboratory staff with adequate equipment and facilities. Methods must be accurate and must be integrated so that the maximum values are obtained with the time and the amounts of samples available.

Recognizing that time had made obsolete much of the literature on human milk, the Nutrition Foundation, Inc., of New York, requested the staff of the Research Laboratory of the Children's Fund of Michigan to reinvestigate the subject and offered partial support for the project. The staffs of the school of nutrition and the department of dairy industry of Cornell University were asked to conduct studies which would provide comparative values for cow's milk.[17] Obviously, procuring data on women while they progress through pregnancy, the puerperium and lactation is a task requiring the joint effort of persons and organizations in several fields. The investigation which provided the material for this group of papers, from one phase, could have been accomplished only through such cooperation.

## PROCEDURE

Samples of milk were obtained from 70 women in the Detroit area who were successfuly nursing infants. Each sample was collected at one of the feeding periods and consisted of the entire amount which could be expressed manually from one or both breasts. The data obtained from this survey represent the composition of milk produced by mothers who were carrying on their usual home activities and eating diets of their own choice.

Intensive studies were made to obtain information on the metabolic processes of milk production, the composition of the total amount of milk secreted daily, and the growth of infants nourished by their mothers' milk. For this investigation, women were selected early in pregnancy, and the course of gestation was followed closely. These women during the period of study were receiving known diets, varying in quantity according to size and appetite but not in quality. They were studied during the first ten days post partum and for five day intervals at various stages of lactation.

The distribution of all the samples analyzed follows:

(*a*) 189 four to eight hour samples obtained in the period from fifteen to three hundred and sixty-two days post partum, from 70 women ingesting self-chosen diets.

(*b*) 175 twenty-four hour collections from 14 women receiving known diets of the same quality.

85 twenty-four hour collections during the first ten days post partum.

90 twenty-four hour collections after the tenth day post partum.

50 twenty-four hour collections during the third month.

25 twenty-four hour collections during the sixth month.

10 twenty-four hour collections during the eighth month.

5 twenty-four hour collections during the tenth month.

18. Beach, E. F.; Bernstein, S. S.; Hoffman, O. D.; Teague, M. D., and Macy, I. G.: Distribution of Nitrogen and Protein Amino Acids in Human and in Cow's Milk, ibid. **139**:57-63 (May) 1941. Beach, E. F.; Bernstein, S. S., and Macy, I. G.: Intake of Amino Acids by Breast Milk-Fed-Infants and Amino Acid Composition of Cow's Milk and Human Milk, J. Pediat. **19**:190-200 (Aug.) 1941.

For medical aspects of the program the collaboration of the Henry Ford Hospital was procured through Dr. J. P. Pratt, chief of the department of obstetrics, who carried the burden, superimposed on the demands of wartime practice, of selecting the subjects, providing meticulous prenatal care, procuring additional information during pregnancy (by roentgen study of long bones, basal metabolism tests and urologic and hematologic examinations) and giving close medical supervision during confinement and the postpartum period.[19] Dr. B. M. Hamil, of the pediatric staff of the hospital, assumed responsibility for the infants while they were in the nursery and throughout the studies, providing medical care and obtaining data on the blood, roentgenograms of bone structure and anthropometric measurements similar to those obtained in a previous study of infant growth.[20]

A complete collection of all milk in the breast was essential to the investigation, for volume and composition vary not only among subjects but in the same subject during a day,[12] and the total milk volume from one breast may not be identical with that from the other, though the relative percentage composition is the same. Manual expression is the only method known to empty the breast completely. Mrs. Velma Davies, a nurse whose interest in breast feeding had led her to develop a technic of manual expression and reestablishment of milk flow[21] based on the work of Sir Truby King, M.D., of New Zealand, was granted a leave of absence by the Visiting Nurse Association of Detroit, so that she could participate in the investigation.[22]

The samples for the survey were obtained from women contacted through the pediatrician and the Visiting Nurse Association of Detroit and did not represent any special race or class. All of the women and their infants were apparently healthy, and the samples were obtained in their homes at feeding times convenient for them. Since some of the collections supplied were the night accumulation of milk, and other collections represented only the interval between two feedings during the day, the samples analyzed represent the excretion in four to eight hours.

In regard to the studies planned to provide information on the production of colostrum, immature milk and mature milk, each subject was carefully selected, and every effort was made to insure accurate laboratory results without interfering with the medical management of the mother and child. Since only one obstetric patient could be handled in any period, an effort was made to select subjects who would deliver in different months. Tentative selection of a subject for the study was made from the history obtained and the examinations made during the woman's first appointment with the obstetrician. Multiparas were selected who were adequate nursers, sound of health, from good environments, emotionally well balanced and possessed of sufficient intelligence to cooperate. Good health was ascertained by careful surveys of their previous records, complete physical examinations and laboratory studies. Good environment was necessary, so that the mother would not be overworked, would have ample rest and be free from worry. Emotional stability was essential to insure a supply of milk from mothers who would not be upset by the conditions imposed by the study. Intelligence was needed for understanding the requirements of the work being done. In the third or fourth month of pregnancy the selection was confirmed and cooperation of the patient assured. At this time the basal metabolic rate was determined and roentgenograms were made of the long bones. At that and each subsequent antepartum visit specimens were obtained for urologic and hematologic examinations.

After one of the subjects was admitted to the hospital, close contact was maintained between the laboratory and the hospital. The patient was cared for in a private or semiprivate room in the obstetric division and was constantly attended by a special nurse. During the day one nurse (V.D.) expressed the milk, and another registered nurse provided nursing care and supervision. The night nurse was able to give all necessary nursing care and collect the milk at the usual periods. Two members of the laboratory staff, in collaboration with the hospital's dietitian,[23] supervised the preparation of meals for the patient, the preparation of duplicates for analysis and the handling of the twenty-four hour accumulations of milk and urine. The diet provided meals typical of those given to patients in the obstetric department of the hospital and was the same qualitatively for every subject, but the quantities of foods were adjusted to the appetite of the mother. Details of the diets given the mothers and of the collection and preparation of the foods and the milk are given in another paper of this series.[24] A member of the obstetric staff procured a sample of blood from the umbilical cord and a member of the laboratory staff, after delivery and before discharge of the mother from the hospital (ten days), obtained a sample of blood from the mother's finger and another sample from the infant's toe for the determination of vitamin C.

## SUMMARY

This paper provides the background for the presentation of the results of a comprehensive study of the secretion of human milk and the composition of human and of bovine milk from

19. Assistance in providing special nursing service during the studies in the hospital was given by Miss Elizabeth Moran, R. N., director of nurses, Henry Ford Hospital, Detroit.

20. Poole, M. W.; Hamil, B. M.; Cooley, T. B., and Macy, I. G.: Stabilizing Effect of Increased Vitamin B ($B_1$) Intake on Growth and Nutrition of Infants, Am. J. Dis. Child. **54**:726-749 (Oct.) 1937; Addition of Vegetable Soup and Strained Vegetables to Diets of Artificially Fed Infants, ibid. **55**:1158-1175 (June) 1938. Slyker, F.; Hamil, B. M.; Poole, M. W.; Cooley, T. B., and Macy, I. G.: Relationship Between Vitamin D Intake and Linear Growth in Infants, Proc. Soc. Exper. Biol. & Med. **37**:499-502 (Dec.) 1937.

21. Davies, V.: Human Milk Studies: XXI. A Simple Technique for the Manual Expression of Mothers' Milk, Am. J. Dis. Child, this issue, p. 148.

22. Miss Emily Sargent, R.N., director of the Visiting Nurse Association of Detroit, gave Mrs. Velma Davies permission to join the staff of the Research Laboratory of the Children's Fund of Michigan during this study and secured many of the subjects for the survey.

23. Mrs. Annie Lou Wertz, B.S., dietitian of Henry Ford Hospital, Detroit, aided in planning the dietary and securing the food for the women while they were in the hospital.

24. Kaucher, M.; Moyer, E. Z.; Richards, A. J.; Williams, H. H.; Macy, I. G., and Wertz, A. L.: Human Milk Studies: XX. The diet of Lactating Women and the Collection and Preparation of Food and Human Milk for Analysis, Am. J. Dis. Child., this issue, p. 142.

e point of view of physiology. The nutritional nplications of breast feeding are discussed, gether with the requirements for obtaining alid data from studies of lactating women and uman milk. The present series of papers on e vitamins in milk will be followed by pub-cations giving (*a*) the proximate composition, ith the mineral and trace element contents, of uman and of bovine milk, (*b*) the dietary and etabolic phases of the studies of the selected others, and (*c*) the growth and skeletal matura-on of the infants.

660 Frederick St.

ACKNOWLEDGMENTS

Valuable suggestions for the coordination and presentation of the data from this series of studies resulted from a special conference held at the Research Laboratory of the Children's Fund of Michigan on Nov. 4, 1944. Those participating with the staff of the laboratory were: Lloyd Kemp, M.D., one of the trustees of the Children's Fund of Michigan; L. A. Maynard, Ph.D., director of the school of nutrition of Cornell University; C. G. King, Ph.D., scientific director, Nutrition Foundation, Inc., New York; J. P. Pratt, M.D., and B. M. Hamil, M.D., of the Henry Ford Hospital; Otto Grob, M.D., Woman's Hospital, Detroit; Emily Sargent, R.N., director, and Velma Davies, R.N., of the Visiting Nurse Association, Detroit; Claire Y. Gunn, Mother's Milk Bureau of Detroit; Clement A. Smith, M.D., medical director, Children's Hospital of Michigan, Detroit.

# HUMAN MILK STUDIES

## XX. THE DIET OF LACTATING WOMEN AND THE COLLECTION AND PREPARATION OF FOOD AND HUMAN MILK FOR ANALYSIS

MILDRED KAUCHER, M.S.; ELSIE Z. MOYER, M.S.; ALLEN J. RICHARDS, B.S.; HAROLD H. WILLIAMS, PH.D.; ANNIE LOU WERTZ, B.S., AND ICIE G. MACY, PH.D.

DETROIT

Food is necessary for health and well-being at all times throughout life, but dietary quality and quantity have special significance when the body is subject to the augmented physiologic activities of growth, reproduction, or repair following injury or disease. As the science of nutrition has developed, there has been demonstrated a need for fuller knowledge of the food requirements of man at all stages of life. The war accentuated the need for the application of the newer knowledge of nutrition to the fighting and production forces, and the shortages resulting from the demands of war reemphasized the lack of sufficient knowledge of food requirements and of how these may be most efficiently and effectively met. Until recently, knowledge of the nutrition of man was developed in large measure through animal experimentation. Man's response to foods, however, may not be the same as those of animals, owing to species differences and environmental factors. Much remains to be learned about the food requirements of man during growth and childbearing, for the improvement of the health and well-being of the race.

Food technology has indicated some of the chemical changes that alter the nutritive value of food. Until more complete knowledge is available on the extent of the changes occurring in foods consequent to ordinary procedures of harvesting, marketing, preparing and serving, it will not be possible to meet food requirements exactly. Fruits or vegetables of the same variety differ in composition, depending on the amount of moisture available, the type of soil in which they are grown, their geographic origin, their ripeness and the modes of marketing and storage. Because of the many factors that influence nutritive value, new emphasis must be placed on determining the nutriments in the foods as they are consumed.

There is a dearth of precise information on the food eaten by nursing women who are successfully feeding their babies and at the same time preserving their own body tissues and maintaining buoyant health. Records of food consumption have been kept during the lying-in period,[1] but seldom have they been continued after this period.[2] To our knowledge, in no instance has the vitamin content of the food eaten been determined quantitatively for women as they progress through lactation. Such a study is a contribution to fundamental information on the dietary requirements of women and is a guide for accurate planning of dietary allowances that will promote the health and the welfare of mothers and babies. This paper records the vitamin contents of planned dietaries eaten by a controlled group of women during studies of lactation and of the composition of human milk from the point of view of physiology.[3] It describes also the collection and preparation of foods and milk for analysis.

### PROCEDURE

During their ten day stay in the hospital after their babies were born, the mothers received known diets, and complete collections of food, urine and milk were made for analyses to determine vitamin, fat, protein and mineral contents and energy value. Under comparable conditions of control and collection, the same women were studied for five day periods in their homes at intervals during lactation.[3] In all studies the

From the Research Laboratory, Children's Fund of Michigan (M. Kaucher, E. Z. Moyer, A. J. Richards, H. H. Williams and I. G. Macy) and the Henry Ford Hospital (A. L. Wertz).

1. Adair, F. L.: Influence of Diet on Lactation, Am. J. Obst. & Gynac. **9**:1-16 (Jan.) 1925. Hoobler, B. R.: The Effect on Human Milk Production of Diets Containing Various Forms and Quantities of Protein, Am. J. Dis. Child. **14**:105-112 (Aug.) 1917; Problems Connected with the Collection and Production of Human Milk, J. A. M. A. **69**:421-429 (Aug. 11) 1917. Bell, M. Studies on the Composition of Human Milk, J. Biol. Chem. **80**:239-247 (Nov.) 1928.

2. Shukers, C. F.; Macy, I. G.; Donelson, E.; Nims, B., and Hunscher, H. A.: Sources of Nutrients Chosen by Women During Pregnancy, Lactation and Reproductive Rest, J. Am. Dietet. A. **7**:235-251 (Dec.) 1931. Food Intake in Pregnancy, Lactation and Reproductive Rest in the Human Mother, J. Nutrition **4**:399-4 (Sept.) 1931.

3. Macy, I. G.; Williams, H. H.; Pratt, J. P., and Hamil, B. M.: Human Milk Studies: XIX. Implications of Breast Feeding and Their Investigation, Am. J. Dis. Child., this issue, p. 135.



TABLE 1.—*Foods Used in the Planned Dietaries of the Nursing Mothers*

| Basic Food Group | Day 1 | Gm. | Day 2 | Gm. | Day 3 | Gm. | Day 4 | Gm. | Day 5 | Gm. |
|---|---|---|---|---|---|---|---|---|---|---|

TABLE 1.—*Foods Used in the Planned Dietaries of the Nursing Mothers*

| Basic Food Group | Day 1 | Gm. | Day 2 | Gm. | Day 3 | Gm. | Day 4 | Gm. | Day 5 | Gm. |
|---|---|---|---|---|---|---|---|---|---|---|
| I | Peaches, canned | 50 | Asparagus, canned | 50 | Carrots | 80 | Peaches, canned | 50 | Peas, canned | 100 |
| | Pea purée | 38 | | | Peas, canned | 100 | Asparagus, canned | 40 | Spinach | 100 |
| | Green beans, canned | 100 | | | Apricots, canned | 100 | Wax beans, canned | 100 | Carrots | 20 |
| | | | | | | | Spinach purée | 38 | | |
| II | Orange juice | 200 | Orange sections | 100 | Strawberries, canned | 100 | Orange juice | 200 | Lemon juice | 5 |
| | | | Tomatoes | 100 | Lemon juice | 5 | Tomatoes | 80 | Tomato juice | 41 |
| | | | | | Tomato juice | 150 | Raspberries, canned | 100 | | |
| III | Potatoes | 200 | Potatoes | 200 | Potatoes | 100 | Potatoes | 100 | Potatoes | 100 |
| | Lettuce | 20 | Lettuce | 120 | Celery | 25 | Lettuce | 100 | Prunes | 100 |
| | Beets, canned | 100 | Apple sauce | 100 | Corn, canned | 100 | | | Lettuce | 40 |
| | Celery | 30 | Pears, canned | 100 | Lettuce | 20 | | | | |
| | Cherries, canned | 100 | | | | | | | | |
| IV | Milk | 1,148 | Milk | 1,015 | Milk | 1,097 | Milk | 1,055 | Milk | 1,101 |
| | Cream, 20% | 108 | Cream, 20% | 100 | Cream, 20% | 70 | Cream, 20% | 138 | Cream, 20% | 91 |
| | Cheese | 49 | Ice cream | 100 | Cottage cheese | 20 | Cheese | 17 | Ice cream | 100 |
| V | Steak | 65 | Meat patty | 90 | Chicken breast | 60 | Steak | 80 | Liver | 50 |
| | Eggs | 65 | Ham | 30 | Salmon | 68 | | | Chicken breast | 60 |
| | | | Eggs | 49 | Eggs | 62 | | | Coconut | 9 |
| | | | | | | | | | Eggs | 66 |
| VI | Bread, whole wheat | 120 | Bread, whole wheat | 120 | Bread, whole wheat | 120 | Bread, whole wheat | 120 | Bread, whole wheat | 120 |
| | Flour (enriched) | 3 | | | Pettijohn's | 150 | Flour (enriched) | 1 | Cream of wheat (enriched) | 150 |
| | Oatmeal | 150 | | | | | | | | |
| VII | Butter | 53 | Butter | 50 | Butter | 45 | Butter | 45 | Butter | 47 |
| Energy, etc. | Melba toast | 5 | Rice Krispies | 20 | Bread crumbs | 6 | Corn flakes | 20 | Bread crumbs | 7 |
| | Sugar | 18 | Bacon | 30 | Sugar | 10 | Macaroni (raw) | 19 | Jelly | 20 |
| | | | Jelly | 20 | Butterscotch pudding powder | 21 | Bacon | 20 | Sugar | 21 |
| | | | Sugar | 10 | Sweet pickle | 15 | Marmalade | 20 | Bacon | 20 |
| | | | Chili sauce | 20 | Stuffed olive | 18 | Sugar | 10 | Jello powder (powdered gelatin) | 25 |
| | | | Mayonnaise | 10 | | | Mayonnaise | 20 | Mayonnaise | 15 |
| | | | | | | | Jello powder (powdered gelatin) | 25 | | |

same staff members were responsible for control of the mothers' diets, the sampling of foods, the collection of the total amount of urine excreted daily and the total amount of milk secreted, the procuring of blood samples by peripheral puncture of the skin of a finger of the mother or a toe of the infant for hematologic examination and determination of ascorbic acid. The diets were essentially the same qualitatively in the hospital and the home, and the procedures of collection and preparation were kept constant.

The menus on which the diets were based were typical of menus for obstetric patients in the hospital and were planned by the dietitian (A. W.). The diets included the same menus for respective days of each five day period, but differences in size and in appetite made quantitative changes necessary. The diet was adjusted to the various appetites by giving one-fifth more of each food (except milk as a beverage) to those who were not satisfied with the diet and one-fifth less to those who found the meals too filling. While the adjusted diets were, in general, qualitatively equivalent according to dietary standards, the analytic values include slight variations produced by a subject's failure to eat all the food of a meal or by an aversion to a food.

The menus were composed of simple foods, which were served in a variety of ways to make attractive, palatable meals. Canned fruit, ice cream or custard was used for dessert. Meat, ham, fish or poultry was served at least once a day and sometimes twice. Liver was served once in each five day period. Although eggs were not given on the fourth day, the average consumption was 1 egg per day. Orange juice, tomato juice, apple sauce or prunes were the breakfast fruits, while potatoes, lettuce and two or three green or yellow vegetables were on the fare daily. Fifty to 60 per cent whole wheat bread was served, and cooked whole grain cereals were included in three of the five breakfasts. Well over 1 quart (about 1,000 cc.) of milk was consumed daily, with generous servings of cream, cheese and butter. Coffee, tea and cocoa were not on the standard diet, because of complications caused in the analysis for one of the vitamins.

Table 1 shows the foods used each day of a five day period, listed according to the "Seven Basic Food Groups."[4] The same recipes were used in preparing corresponding meals for all the women, but they were increased or decreased 10 to 20 per cent to adjust the quantity of food to the size and the appetite of the subject. The quota of milk as a beverage was kept constant at 1,000 cc. per day for all women. Eggs also were not increased or decreased except as they were used in recipes from which weighed servings were made. The majority of the subjects ate all of the foods served them, but if a woman did not feel like eating all of her meal, as occasionally happened, the leftovers were weighed and subtracted from the intakes and equal amounts were removed from the duplicate meal for analysis. The women cooperated by eating such foods as could not be weighed back accurately (eggs scrambled with chopped ham, soft boiled eggs, vegetables to which butter had been added, jello salad, macaroni and cheese, and any salad or vegetable to which salt had been added from the weighed saltcellar). Few food idiosyncrasies were encountered. However, there was a woman who never tasted cottage cheese and did not want it included in the menu. Another would not eat mayonnaise. An individual saltcellar was weighed at the beginning and at the end of each five day period to determine the amount of salt used by the subject in addition to that in the cooked food. The menus given in table 2 were devised to afford a variety of attractive meals for the women during each five day period.

Certain general procedures for selection, preparation and weighing of food were followed: Fruits were pitted before weighing. Five grams of butter was added to certain vegetables after they were weighed. The amount of juice added to each serving of fruit or vegetable was kept at 5 Gm.; that is, a 100 Gm. serving of green beans included 95 Gm. of drained beans and 5 Gm. of juice. Efforts were made to heat all canned vegetables approximately ten minutes before serving. Market milk was used: standard milk, homogenized vitamin D milk or Jersey milk, whichever the mother was accustomed to getting.

TABLE 2.—*Daily Menus for Five Day Periods*

1. **Breakfast:** Orange juice, oatmeal, soft boiled egg, toast, whole wheat bread, butter, sugar, cream, milk.
   **Dinner:** Cream of pea soup, steak, cubed potatoes, canned green beans, whole wheat bread, butter, celery heart, custard, milk.
   **Supper:** Welsh rarebit on Melba toast, plain boiled potatoes, canned beets, whole wheat bread, butter, salad of canned peach on lettuce, canned white cherries, milk.
2. **Breakfast:** Applesauce, Rice Krispies, bacon, toasted whole wheat bread, butter, jelly, cream, sugar, milk.
   **Dinner:** Ground beef patty with chili sauce, mashed potatoes, canned asparagus, whole wheat bread, butter, head lettuce with mayonnaise, vanilla ice cream, milk.
   **Supper:** Scrambled egg and ham, baked potato, stewed tomatoes, whole wheat bread, butter, salad of orange sections on lettuce, canned pears, milk.
3. **Breakfast:** Tomato juice, Pettijohn's, soft-boiled egg, toasted whole wheat bread, butter, cream, sugar, milk.
   **Dinner:** Chicken breast, mashed potato, canned peas, whole wheat bread, butter, salad of apricot halves with cottage cheese on lettuce, butterscotch pudding, cream, milk.
   **Supper:** Salmon loaf, lemon juice, stewed carrots, canned corn, whole wheat bread, butter, relish plate of celery, pickles and stuffed olives, canned strawberries, milk.
4. **Breakfast:** Orange juice, corn flakes, bacon, toasted whole wheat bread, butter, marmalade, sugar, cream, milk.
   **Dinner:** Steak, potato balls, canned wax beans, whole wheat bread, butter, head lettuce with mayonnaise, gelatin with canned peach, milk.
   **Supper:** Cream of spinach soup, macaroni and cheese, stewed tomatoes, whole wheat bread, butter, asparagus salad on lettuce with mayonnaise, canned raspberries, milk.
5. **Breakfast:** Stewed prunes, Cream of Wheat, soft-boiled egg, toasted whole wheat bread, butter, jelly, sugar, cream, milk.
   **Dinner:** Liver with bacon, baked potato, spinach, lemon juice, lime jello with carrot strips, lettuce and mayonnaise, whole wheat bread, butter, vanilla ice cream, milk.
   **Supper:** Cream of tomato soup, chicken breast on lettuce, canned peas, whole wheat bread, butter, coconut custard, milk.

   (Additional milk was served at midmorning, midafternoon and before retiring.)

In weighing stuffed olives, the pimento and the meat portion were weighed separately. Potatoes varied with the season; some were new, some old. Baked potatoes were scooped from the shell and weighed. Mashed potatoes were made in small quantities, with approximately the same proportions of potatoes, milk and butter being used. In spinach and pea soup, canned, strained vegetables were used; tomato juice was used to make tomato soup. Apple sauce was taken from the can or was made from fresh apples the night before and kept in the ice box. After the meats had been cooked, fat and gristle were removed before the portions were weighed to provide more accurate sampling of the lean meat.

In boiling eggs, two eggs of similar weights were boiled three or four minutes and then weighed minus their shells, one for the patient and one for the laboratory sample. Duplicate scrambled egg and chopped ham portions were cooked separately. Two molds

4. National Wartime Nutrition Guide, United States Department of Agriculture, War Food Administration, Nutrition and Food Conservation Branch, July 1943.

elatin salad were made, the ingredients being weighed eparately. Bread crumbs were made from commercial Melba toast (a thin, completely dry toast). Medium-oasted bread was weighed before and after toasting. To vercome seasonal scarcity and variation in fresh prod-cts, canned fruits and vegetables were used almost xclusively, except for oranges and lemons, and were urchased in case lots.

For every meal, after each food was prepared two dentical portions were weighed. One of the portions as served to the mother; the other was used for aboratory analysis. For the latter purpose, the liquid foods ere poured into an amber glass jar containing 5 cc. of enzene. The solid foods were placed in another amber lass jar containing 5 cc. of benzene, and after each meal bout 80 cc. of 5 per cent acetic acid was poured over he contents. The jars in which the day's collections f liquid and solid foods were accumulated were kept nder refrigeration until they were transported to the aboratory the following morning.

For the determination of ascorbic acid, one tenth of he amount of each vitamin C–containing food eaten was eighed into a jar and an equal portion of 6 per cent etaphosphoric acid solution added. Only food definitely nown to lack or to contain inappreciable amounts of his vitamin, such as bread, cereals, macaroni and cheese, ugar, jello, butter, mayonnaise, bacon and eggs, were itted from the sample. The jar was kept in an ice ox until taken to the laboratory the following morning, here the material was immediately homogenized in a Waring blendor and analyzed.

## RESULTS AND COMMENT

Protein (nitrogen), energy, ascorbic acid, itamin A, carotene, thiamine, riboflavin, nico-inic acid, calcium pantothenate and biotin were etermined in the dietaries used during 30 five ay studies of 11 nursing women. The average aily intakes are shown in table 3, together with he allowances for lactating women recommended y the Food and Nutrition Board of the National Research Council.

The intake of protein averaged 109.5 Gm. aily. It was calculated that 77 per cent of the rotein in the standard diet was from animal ources, while 23 per cent was of vegetable rigin. The diet was liberally supplied with eat, eggs and milk and the average daily ntake of protein was 10 per cent above the ecommended allowance of 100 Gm.

The average daily intake of energy, 2,936 alories, was 98 per cent of the recommended ,000 calories.

The intake of total vitamin C averaged 125 mg., hich is 83 per cent of the advocated amount f 150 mg.

The diet was rich in vitamin A and carotene, ontributed largely by liver, spinach, carrots, pricots, butter, cream, milk and eggs. The verage daily intake of vitamin A was 1,455 icrograms; that of carotene, 6,248 micrograms. ecause some doubt exists as to the factor best uited for converting micrograms of carotene into micrograms of vitamin A, these nutriments have been reported as separate constituents. However, the 1,455 micrograms of vitamin A alone represents a large proportion of the recommended intake of 1,860 micrograms. There was available, in addition, the vitamin A derived from 6,248 micrograms of carotene.

The daily intake of thiamine averaged 1.23 mg., derived mainly from milk, whole wheat bread and cereals, eggs, meats, potatoes, peas and orange juice. The value for this vitamin was only 62 per cent of the recommended allowance of 2 mg.

Nicotinic acid, too, was below the recommended allowance, the average daily intake of 16.50 mg. being 82 per cent of the recommended 20 mg. Foods contributing the largest proportion of this vitamin in this diet were peas, potatoes, meat, whole wheat bread, cereals and milk.

The daily average intake of 3.14 mg. of riboflavin was 5 per cent over the allowance of 3 mg. recommended. The fact that the average intake of riboflavin in the diet was somewhat in excess of the recommended allowance is largely due to the liberal use of milk, since each woman received slightly over a quart of milk daily. It would indeed be difficult for the lactating mother to receive the recommended amount of riboflavin without a generous allotment of milk.

The fact that the average values determined for ascorbic acid, thiamine and nicotinic acid were considerably lower than those recom-

TABLE 3.—*The Controlled Nursing Mothers' Average Daily Intakes of Protein, Energy and Vitamins, Determined by Analysis of the Food as Eaten*

| | | Intake Obtained by Analysis of Food as Eaten | |
|---|---|---|---|
| | Recommended Allowance * | Total † | Per Cent of Recommended Allowance |
| Protein, Gm. | 100 | 109.5 | 110 |
| Energy, calories | 3,000 | 2,936 | 98 |
| Ascorbic acid, mg. | 150 | 125 | 83 |
| Vitamin A ‡, micrograms | 1,860 | 1,455 | .. |
| Carotene, micrograms | .... | 6,248 | .. |
| Thiamine, mg. | 2.0* | 1.23 | 62 |
| Riboflavin, mg. | 3.0 | 3.14 | 105 |
| Nicotinic acid, mg. | 20* | 16.50 | 82 |
| Calcium pantothenate, mg. | .. | 7.82 | .. |
| Biotin, micrograms § | .. | 56.24 | .. |

* Recommended Dietary Allowances, Food and Nutrition Board, Reprint and Circular, Series 115, National Research Council, 1943. The values for thiamine and nicotinic acid were lowered in the 1945 revision of the "Recommended Allowances."

† This represents an average of results from 25 to 30 five day studies.

‡ The number of micrograms was obtained by dividing the number of U. S. P. or international units (8,000) by factor 4.3. Lesher, M.; Brody, J. K.; Williams, H. H., and Macy, I. G.: Human Milk Studies: XXVI. Vitamin A and Carotenoid Contents of Colostrum and Mature Human Milk, Am. J. Dis. Child., this issue, p. 182).

§ Since the assays for this vitamin were made on weak acid (tenth normal hydrochloric acid) extracts of the food composite, the average value given does not include the firmly bound biotin present in considerable amounts in certain foods, notably meats. This bound biotin is freed only by one to three hour hydrolysis with twice to four times normal acids.

mended for lactating women emphasizes in part the amounts of these vitamins lost in preparation and cooking. In another paper [5] we have shown that for these vitamins the values calculated from recognized food tables are higher than the values obtained by analysis. The analytic data represent "food as eaten," which had gone through the processes of marketing, storage, preparation, cooking and serving, wherein losses of vitamins undoubtedly occurred. That the values for ascorbic acid, thiamine and nicotinic acid obtained by analysis were lower than the calculated values and lower than the recommended allowances also lends added emphasis to the need for determining values of foods as these are consumed. There is some evidence that the allowances recommended for thiamine and riboflavin may be too high. Keys,[6] Melnick [7] and some of the English authorities—for example, McRae [8] have tended toward this view. Morgan [9] reported that 2 lactating women had dietary intakes of thiamine of from 1.05 to 1.65 mg. per day.

The data from the analyses described provide average values of which only those for protein, vitamin A and riboflavin exceed the recommended allowances. However, the weights of the women studied varied from 100 to 200 pounds (45.5 to 90.5 Kg.), which was not considered in computing the average values. The women's appetites were completely satisfied by the diets, their body weights were maintained, and subsequent papers in this series demonstrate that their milk contained greater concentrations of some of the vitamins than did the milk of women eating self-chosen diets.

Table 4 shows the average volumes of milk produced daily by the women receiving the diets described on the preceding pages.

*Preparation of Food Composites for Analysis.*—A large electric-powered meat grinder that was tin coated except for the grinding surfaces was used to grind the composite of solid food. Then the liquid food composite was poured through the grinder, followed by washings (distilled water) of the jars which had contained the composites. Finally the grinder was rinsed with additional distilled water. All the material was caught in a 12 liter porcelain evaporating dish, which had been cleaned, rinsed with distilled water and weighed.

After the total weight had been recorded, the material was mixed well with an enamelware dipper and one fifth of it transferred to a wide-mouthed, glass-stoppered 4 liter bottle and held under refrigeration. At the end of the five day period, the bottle contained one fifth of each day's intake.

After this composite had been thoroughly mixed, samples for analysis were removed from it as follows:

A 400 Gm. sample was removed, put through a Waring blender and used in the analyses for the B vitamins. A 40 to 60 Gm. portion of the composite was weighed into each of three or four glass-stoppered, round bottom flasks. After the weights of these had been recorded, the samples were used for determinations of vitamin A and carotenoids. A sample consisting of 300 Gm. of the material was placed in a 1 liter beaker and digested on a steam bath with 300 cc. of concentrated sulfuric acid for three or four days, with occasional stirring to break up the lumps and insure complete decomposition. The digest was made to volume, and aliquots were taken for the determination of nitrogen by the macro-Kjeldahl method.

TABLE 4.—*Average Daily Volumes of Milk Secreted by the Women Receiving Known Diets*

| | Stage of Lactation, Day | Subjects | 24 Hour Collections | Volume per Day | |
|---|---|---|---|---|---|
| | | | | Range, Cc. | Mean, Cc. |
| Hospital | 1 | 6 | 6 | 6-82 | 39 |
| | 2 | 6 | 6 | 56-385 | 167 |
| | 3 | 6 | 6 | 353-870 | 618 |
| | 4 | 6 | 6 | 547-1,413 | 914 |
| | 5 | 6 | 6 | 560-1,471 | 1,022 |
| | 6 | 6 | 6 | 663-1,782 | 1,130 |
| | 7 | 9 | 9 | 781-1,630 | 1,112 |
| | 8 | 7 | 7 | 688-1,895 | 1,152 |
| | 9 | 7 | 7 | 794-1,828 | 1,256 |
| | 10 | 6 | 6 | 797-1,872 | 1,316 |
| Home | 45-74 | 5 | 23 | 234-979 | 620 |
| | 75-99 | 6 | 27 | 544-1,083 | 820 |
| | 144-165 | 3 | 15 | 272-1,000 | 635 |
| | 173-191 | 2 | 6 | 205-868 | 368 |
| | 204-208 | 1 | 5 | 864-938 | 913 |
| | 239-263 | 2 | 10 | 610-772 | 678 |
| | 302-306 | 1 | 5 | 383-414 | 394 |

Into each of four weighed 250 cc. centrifuge bottles was placed 100 Gm. of the food composite. After the exact weights of the wet samples had been recorded, the bottles were placed in a deep freeze (— 50 F.) where they were held prior to drying by the cryochem process ("desiccation *in vacuo* from the frozen state by means of chemicals" [10]).[11] The dried samples were transferred to brown bottles with screw caps and stored in a desiccator.

The remainder of the material representing the diet for five days was placed in two weighed 1 liter evaporating dishes and the wet weights recorded. One hundred cubic centimeteters of alcohol U. S. P. was added and stirred into each sample before the sample was covered with cotton gauze to prevent contamination with dust and placed in a steam oven to dry at 60 C. The drying

5. The Food Requirements of Lactating Women, J. Am. Dietet. A., to be published.

6. Keys, A.; Henschel, A. F.; Mickelson, O., and Brozek, J. M.: The Performance of Normal Young Men on Controlled Thiamine Intakes, J. Nutrition **26**: 399-415 (Oct.) 1943.

7. Melnick, D.: Vitamin $B_1$ (Thiamine) Requirement of Man, J. Nutrition **24**:139-151 (Aug.) 1942.

8. Macrae, T. F.; Barton-Wright, E. C., and Copping, A. M.: The Riboflavin Content of Food Served in Royal Air Force Messes, Biochem. J. **38**:132-135, 1944.

9. Morgan, A. F., and Haynes, E. G.: Vitamin $B_1$ Content of Human Milk as Affected by Ingestion of Thiamine Chloride, J. Nutrition **18**:105-114 (Aug.) 1939.

10. Flosdorf, E. N., and Mudd, S.: An Improved Procedure and Apparatus for Preservation of Sera, Microörganisms and Other Substances—The Cryochem Process, J. Immunol. **34**:469, 1938.

11. Teague, D. M.; Galbraith, H.; Hummel, F. C.; Williams, H. H., and Macy, I. G.: Effects of Desiccation Procedures on the Chemical Composition of Feces, Urine and Milk, J. Lab. & Clin. Med. **28**:343-348 (Dec.) 1942.

usually required a week to ten days before a constant weight was attained. The dried samples were combined, ground to powder, placed in brown bottles, labeled, capped and stored in a large desiccator.

*Milk Collection and Preparation.*—In all the studies the mother's milk was expressed manually. As regards the women who were studied in the hospital and at their homes while receiving known diets both breasts were completely emptied at four hour intervals. For all patients who delivered between noon and midnight, the first twenty-four hour milk collection was started the morning after parturition; for those who delivered their babies between midnight and noon, the day of delivery was the first day of the study and therefore the first day of collection of the milk. Thus the interval between delivery and the first attempt to express colostrum ranged from eight to seventeen hours. Colostrum was obtained from 1 mother eight hours after parturition, but 2 mothers with whom manual expression first was attempted ten and twelve hours after delivery did not have colostrum until twenty-two and twenty-four hours post partum.

Aseptic technic was used in expressing all milk into sterile receptacles.[12] Small quantities of milk were collected directly into either a 200 cc. beaker, a porcelain cup or an aluminum measuring cup and transferred into a 500 cc. graduate. As soon as the collection was complete, the volume was recorded, and the milk stirred with a sterile glass rod. In the controlled studies, an aliquot of one tenth to one one hundredth of the total was transferred into a brown glass bottle with a plastic screw cap. An equal amount of 4 to 6 per cent metaphosphoric acid solution was added with a pipet immediately, to preserve the ascorbic acid. From the remainder of the collection an aliquot was poured into a 1 liter amber glass bottle with a tight cover. The aliquot was the amount which with a similar aliquot from each of the six expressions during the twenty-four hours would provide a total of 250 cc. for analysis. The bottles were kept in a refrigerator between collections. The excess of milk over the amount needed for analysis was fed to the infant by bottle.

The survey samples represented only one milk expression (in some cases two). After the volume had been recorded, 10 cc. was measured into a bottle fitted with a plastic screw cap and containing 2.4 mg. of solid metaphosphoric acid. The remainder was poured into an amber glass bottle, and both collections were transported immediately to the laboratory.

On arrival at the laboratory, the volumes of the milk samples were checked against the previously recorded figures, and aliquots were removed for determinations of the various vitamins. When analyses for the B complex vitamins could not be made immediately, the required amount of milk was held at — 50 F. From the twenty-four hour collections obtained in the hospital and the homes, aliquots also were taken for the determination of the nitrogen partition.

The remainder of the milk was placed in weighed 250 cc. centrifuge bottles (maximum, 125 cc. per bottle), frozen in a cabinet which maintains a temperature of — 35 to — 50 F. and dried from the frozen state by the cryochem process.[11] During handling and desiccation of the samples every precaution was taken to exclude light in order to avoid destruction of light-labile constituents, particularly riboflavin.

After desiccation, the bottles were reweighed, and the water content and the total solids content of each sample were calculated. The dry milk residues were transferred to amber bottles fitted with screw caps (plastic) and stored in desiccators for determinations of total caloric (energy) value, fat and mineral distribution.

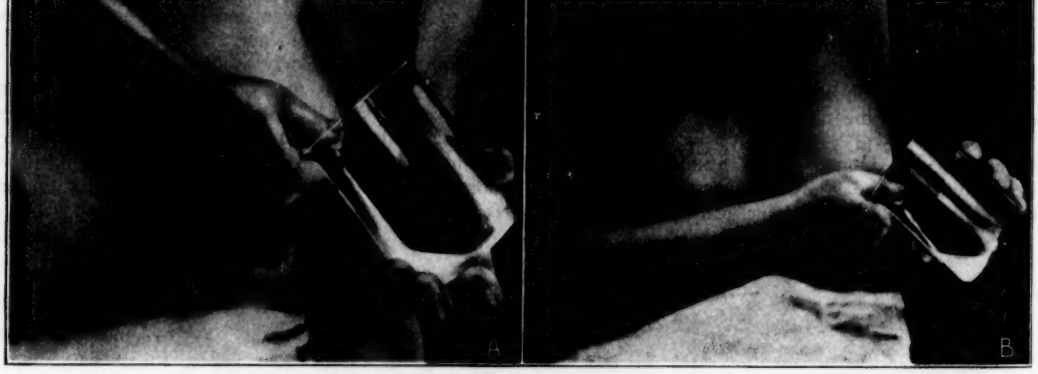
12. Davies, V.: Human Milk Studies: XXI. A Simple Technic for the Manual Expression of Mothers' Milk, Am. J. Dis. Child., this issue, p. 148.

# HUMAN MILK STUDIES

## XXI. A SIMPLE TECHNIC FOR THE MANUAL EXPRESSION OF MOTHERS' MILK

VELMA DAVIES, R.N.
DETROIT

During the period of lactation the milk glands in the breast secrete milk continuously. The fluid fills the network of tiny tubes which converge near the nipple and empty into fifteen to twenty small temporary reservoirs called milk sacs, which are about the size of beans. The milk sacs are under the dark area (areola) which surrounds the nipple, with which they are connected by milk ducts. Not all of the milk sacs are just beneath the skin; therefore, complete emptying of the breast requires gentle pressure on the tissue underlying the entire areola. In nursing, the infant draws the nipple well into his mouth so that his lips compress the entire area behind which the milk sacs lie. There are many conditions which permanently or temporarily prevent a mother from nursing her infant, but most of them are no reason for withholding from the baby the best food possible.[1]



Manual expression of milk by the nurse (*A*) and by the mother (*B*).

Records of manual expression of human milk to relieve pressure within the breast and to stimulate production can be found dating from as far back as 100 A. D. Recent publications directed to doctors, nurses and mothers illustrate methods of manual expression which, because they are difficult to master, have not been used widely. For the past fifteen years I have used a method which is simple, does not require special equipment and can be taught to a mother in a few minutes. With this method the breast can be emptied more completely than with a mechanical or a hand pump, and besides the advantages of ease and speed, it offers less likelihood of contamination as compared with other manual methods. Since the first and the last milk expressed from the breast vary in composition,[2] it is important that manual expression, in addition to stimulating milk flow[3] and obtaining mothers' milk for infants who cannot nurse or be nursed, offers the only means of obtaining a complete collection of the milk secreted in a definite period of time.[4] I have not found this technic described in the available literature.

From the Research Laboratory, Children's Fund of Michigan, and the Visiting Nurse Association.

1. A detailed discussion of the application of the technic of manual expression in stimulating increased secretion of milk and in maintaining and reestablishing lactation, as well as in the assurance of breast milk for infants who cannot be nursed, is being prepared for another journal.

2. Macy, I. G.; Nims, B.; Brown, M., and Hunscher, H. A.: Human Milk Studies: VII. Chemical Analysis of Milk Representative of Entire First and Last Halves of the Nursing Period, Am. J. Dis. Child. **42**:569-58 (Sept.) 1931.

3. Macy, I. G.; Hunscher, H. A.; Donelson, E. and Nims, B.: Human Milk Flow, Am. J. Dis. Child. **39**:1186-1204 (June) 1930.

4. Macy, I. G.; Williams, H. H.; Pratt, J. P., and Hamil, B. M.: Human Milk Studies: XIX. Implications of Breast Feeding and Their Investigation, Am. J. Dis. this issue, p. 135.

### DESCRIPTION OF TECHNIC

The only utensils needed are a sterilized cup or beaker ith smooth, rounded edge, a funnel and a container r storage of milk. The hands and the breasts should washed with warm soapy water and dried with rile gauze or towel.

The accompanying illustration shows the position of e hands in relation to the breast when a second per- n is expressing the milk and when the mother is pressing her own milk. The breast is supported with ur fingers of one hand under it and below the eolar tissue; the thumb is above the nipple and well ck on the top of the areola. The cup is held in the her hand, its edge placed against the lower margin the areola and resting against the index finger of the nd grasping the breast.

With the thumb, pressure is applied to the areola, rcing the underlying tissue downward against the in- le wall of the cup. The milk sacs are compressed tween the thumb and the cup and the fluid gently ced through the ducts to the nipple. As the milk emitted, it falls directly to the bottom of the cup thout contact with the surface of the breast or with e hand. Pressure of the thumb is released without anging its position against the breast. (If the thumb permitted to rub against the skin, irritation may occur.) The downward compression strokes are continued at a rate of 30 to 50 per minute.

The exact tilt of the cup and the line of contact between the cup and the areola will vary slightly, depending on the depth of the milk sacs. The proper position causes no pain or tenderness and permits free and rapid expression of the milk. The time required for complete emptying of a breast depends on the skill of the operator, the fulness of the gland and individual characteristics of the structure. In some breasts the ducts leading from the milk sacs to the nipple require more stimulation for relaxation than those in others, and some breatss release milk more readily than others.[5] A breast may yield little milk on the first attempt at manual expression, but with this method any mother can learn, with a small amount of practice, to withdraw her milk. When a mother with a plentiful milk flow must express her entire output, the procedure is difficult until the finger muscles in the forearm have been developed by the unaccustomed activity. However, the muscles usually have ample opportunity to develop as lactation increases. When manual expression is used merely to maintain the flow of milk over short periods, to increase the supply or to reestablish lactation, fatigue is seldom a problem.

---

5. Peterson, W. E.: Lactation, Physiol. Rev. **24**: 340-371 (July) 1944.

# HUMAN MILK STUDIES

## XXII. NICOTINIC ACID, PANTOTHENIC ACID AND BIOTIN CONTENTS OF COLOSTRUM AND MATURE HUMAN MILK

MARGARET N. CORYELL, PH.D.; MARY ELLEN HARRIS, B.S.; SOLOMON MILLER, M.S.; HAROLD H. WILLIAMS, PH.D., AND ICIE G. MACY, PH.D.

DETROIT

That nicotinic acid (niacin) is important in the metabolism of human beings is an established fact supported by intensive experimental studies over many years. The amide of nicotinic acid, nicotinamide, has been shown to be an integral part of the pyridine nucleotide coenzymes that function in the oxidative enzyme systems which are necessary to the cell respiration of the living organism. There also is a possibility that nicotinic acid may have functions besides those involving oxidation systems, since it has been shown that a deficiency may affect the water and electrolyte balances,[1] gastric motility [2] and the metabolism of porphyrins [3] and lipids.[4] However, many experimental results from studies involving the biologic activity of nicotinic acid and its derivatives, especially nicotinamide, are contradictory. Hence, the theories based on them are difficult to coordinate and make any definite statements concerning the physiologic activities of nicotinic acid of doubtful value.

The symptoms of a deficiency of pantothenic acid have been repeatedly demonstrated in various laboratory animals, but no clearcut deficiency has been noted in man. However, low levels of pantothenic acid in the blood have been found to accompany other deficiency states in human subjects, such as pellagra, beriberi and riboflavin deficiency.[5] Since pantothenic acid has been shown to be necessary for the normal grow and metabolism of numerous micro-organis and higher animals, it seems reasonable to assu that man requires an exogenous source of t vitamin. Although the manifestations of a d ficiency of pantothenic acid in animals vary co siderably with the species, the results indica that the deficiency is more marked in the you than in the mature animal and that impairme of growth is frequently one of the major sym toms.[6] It therefore is probable that this vitam is of greater importance to the infant and t growing child than to the human adult.

The importance of biotin in the metabolism the living organism was recognized long bef it was actually identified, but its specific r also is still undetermined. The fact that bio is so widely distributed in natural foodstuffs a that extremely small amounts are physiologica active undoubtedly has been a factor in this la of exact information. As with pantothenic ac a clinical deficiency of biotin has never be described, although in a few isolated cases cutaneous ailments (acne vulgaris) biotin the apy has given curative results.[7] However, e perimental deficiency of biotin has been demo strated frequently in laboratory animals. T produce the deficiency in rats it is necessary incorporate raw egg white or sulfonamide co pounds (sulfaguanidine, succinylsulfathiazol into a biotin-deficient diet. The egg white co tains avidin, a protein which combines wi biotin to form a complex which is not absorb from the intestinal tract. The sulfonamide co pounds apparently inhibit the growth or t metabolism of certain intestinal micro-organis (Bacillus coli), hence their production of biotin

From the Research Laboratory of the Children's Fund of Michigan.

1. Handler, P., and Dann, W. J.: The Biochemical Defect in Nicotinic Acid Deficiency, J. Biol. Chem. **145**:145-153 (Sept.) 1942.

2. Martin, G. J.; Thompson, M. R., and Carvajal-Forero, J.: The Influence of Inositol and Other B Complex Factors upon the Motility of the Gastro-Intestinal Tract, Am. J. Digest. Dis. **8**:290-295 (Aug.) 1941.

3. Spies, T. D.; Bean, W. B., and Stone, R. E.: The Treatment of Subclinical and Classic Pellagra, J. A. M. A. **111**:584-592 (Aug. 13) 1938.

4. Handler, P., and Dann, W. J.: The Inhibition of Rat Growth by Nicotinamide, J. Biol. Chem. **146**: 357-368 (Dec.) 1942.

5. Stanbery, S. R.; Snell, E. E., and Spies, T. D.: A Note on an Assay Method for Pantothenic Acid in Human Blood, J. Biol. Chem. **135**:353-354 (Aug.) 1940.

6. Silber, R. H.: Studies of Pantothenic Acid D ficiency in Dogs, J. Nutrition **27**:425-433 (May) 19

7. Marshall, W.: Further Studies on the Thera of Acne Vulgaris with Modified Liver Extract, J. Inve Dermat. **2**:205-218 (Aug.) 1939.

8. Welch, A. D., and Wright, L. D.: The Role "Folic Acid" and Biotin in the Nutrition of the R J. Nutrition **25**:555-570 (June) 1943.

By incorporating desiccated egg white in a biotin-deficient diet to the extent of 30 per cent of the total calories an experimental deficiency of biotin has been produced in man, and the symptoms have been alleviated by administration of crystalline biotin.[9] Experimental deficiency of biotin in man and animals has shown that the presence of biotin is important to the normal condition of the skin, the hair and the muscles, to the nervous system, to growth, to fat metabolism and to the normal utilization of pantothenic acid.[10] Since biotin is a growth factor for micro-organisms, higher plants and certain animals, it probably is necessary for the normal growth of children.

The literature contains a few data on the nicotinic acid content of human milk; less is known of the concentrations of pantothenic acid and biotin. Investigation of the amounts of these vitamins in the food Nature provides for infants is fundamental to an understanding of the roles of these vitamins in the growth and development of human beings. This paper contributes to that end and gives the results of analyses for the nicotinic acid, the pantothenic acid and the biotin of twenty-four hour collections of milk obtained from women receiving known diets, planned by a dietitian, and of four to eight hour samples from women eating diets of their own choice. Of the milk samples analyzed for nicotinic acid, pantothenic acid and biotin, 73, 62 and 48, respectively, represented complete daily collections of immature milk secreted during the first ten days post partum; 86, 86 and 85, respectively, represented complete daily collections of mature milk secreted from two to ten months post partum. From 181 to 183 samples represented four to eight hours' collection of milk and were obtained at intervals ranging from one month to one year post partum.

9. Sydenstricker, V. P.; Singal, S. A.; Briggs, A. P.; DeVaughn, N. M., and Isbell, H.: Preliminary Observations on "Egg White Injury" in Man and Its Cure with a Biotin Concentrate, Science **95**:176-177 (Feb. 13) 1942.

10. Emerson, G. A., and Keresztesy, J. C.: Biotin Deficiency in the Rat, Proc. Soc. Exper. Biol. & Med. **47**:358-361 (Dec.) 1941. Nielsen, E., and Elvehjem, C. A.: Cure of Paralysis in Rats with Biotin Concentrates and Crystalline Biotin, J. Biol. Chem. **144**:405-409 (July) 1942. Shaw, J. H., and Phillips, P. H.: Pathological Studies of Acute Biotin Deficiency in the Rat, Proc. Soc. Exper. Biol. & Med. **51**:406-407 (Dec.) 1942. Wright, L. D., and Welch, A. D.: Folic Acid, Biotin and Pantothenic Acid Deficiency and the Liver Storage of Various Vitamins in Rats Fed Succinylsulfathiazole in Highly Purified Rations, J. Nutrition **27**:55-66 (Jan.) 1944. Nielsen, E., and Black, A.: Biotin and Folic Acid Deficiencies in the Mouse, ibid. **27**:203-207 (Sept.) 1944.

## METHODS

*General Procedure.*—The tubes used for the assays were bacteriologic culture tubes, 150 by 18 mm. Each tube was calibrated to a volume of 10 cc. Further, all tubes were calibrated for transmittance of light. A dilute ink solution was placed in each tube, and those whose transmittance varied more than ± 1 per cent from the average were discarded.

Stock cultures of Lactobacillus arabinosus and Lactobacillus casei were maintained by monthly transfers to new agar medium of the following composition per hundred cubic centimeters:

| | |
|---|---|
| Yeast dextrose agar (Difco) | 2.7 Gm. |
| Dextrose | 1.0 Gm. |
| Sodium acetate | 1.6 Gm. |
| Salt solution A * | 1 cc. |
| Salt solution B † | 1 cc. |

* Twenty-five grams of potassium phosphate and 25 Gm. of potassium dihydrogen phosphate in 250 cc. of water.

† Ten grams of magnesium sulfate, 0.5 Gm. of sodium chloride, 0.5 Gm. of iron sulfate and 0.5 Gm. of manganese sulfate in 250 cc. of water and 2 to 6 drops of concentrated hydrochloric acid.

Four stab cultures were made in this medium and incubated twenty-four hours at 32 C., the tubes being capped with Parafilm to prevent dehydration, and then stored in the refrigerator. The assay cultures were prepared by transferring material from the agar stab cultures into 10 cc. of a liquid medium of the following composition per hundred cubic centimeters:

| | |
|---|---|
| Peptone (Difco) | 0.5 Gm. |
| Dextrose | 1.5 Gm. |
| Tryptone (Difco) | 0.25 Gm. |
| Yeast extract (Difco) | 0.1 Gm. |
| Sodium acetate | 0.8 Gm. |
| Cystine | 10 mg. |
| Salt solution A | 0.5 cc. |
| Salt solution B | 0.5 cc. |

The $p_H$ of this medium was adjusted to 6.6 with sodium hydroxide. After twenty to twenty-four hours' incubation at 32 C., the cultures were centrifuged, and the supernatant medium was poured off and replaced aseptically with an equal volume of sterile 0.9 per cent sodium chloride solution. The organisms were resuspended in the sodium chloride solution,[11] and from each suspension 1 cc. was transferred aseptically to a tube containing 10 cc. of sterile 0.9 per cent sodium chloride solution. One drop of this diluted culture was used as the inoculum for each assay tube.

Two dilutions of each unknown were made and five levels of each dilution (1 to 5 cc.) were used, a total of 10 tubes.

To each of the assay tubes containing the standard solution of the vitamins, to those containing unknown samples and to two tubes containing water, 5 cc. of basal medium was added and the volume adjusted to 10 cc. with distilled water. The tubes were then plugged and autoclaved fifteen minutes at 15 pounds (6.8 Kg.) of pressure, after which they were cooled, inoculated and incubated at 32 C. The growths in the assay tubes were measured turbidimetrically and titrimetrically. The turbidity was measured with a Cenco-Sheard

11. Instead of starting the assay culture from the agar stab culture each day, one drop of this suspension was transferred to a fresh tube of liquid medium and a new assay culture started for the following day. At least once each week the assay culture should be started from a fresh agar stab culture.

Photelometer[12] or a Coleman Spectrophotometer after twenty to twenty-four hours' incubation at 32 C. The instrument was set to read 100 per cent transmittance with nothing in the path of light. After thorough shaking, the tubes were placed in the instrument, and the percentage transmittance of light by each was recorded. The tubes were then returned to the incubator for an additional forty-eight ± ten hours' incubation at 32 C. After a total incubation time of seventy-two ± hours, the acid produced in each tube was titrated with tenth-normal sodium hydroxide, phenolsulfonphthalein (phenol red) being used as an indicator.

*Calculations.*—The turbidity readings of the standard tubes in terms of percentage transmittance of light were plotted on semilogarithmic graph paper to obtain a standard curve. The titration values were recorded in terms of tenth-normal alkali, and the values for the standard tubes were plotted on ordinary cross-section graph paper to obtain the standard curve of acid production. The turbidity value of the unknown, obtained from the standard turbidity curve, was averaged with the titration value of the unknown, obtained from the standard titration curve, to give the final value. This procedure was followed in the early part of this study, but inasmuch as the values obtained by the two measurements agreed well in most cases, and since neither seemed to offer greater accuracy or uniformity, the turbidimetric measurement alone was used in the latter half of this study, as considerably less time was involved in the turbidimetric measurement than in the titration procedure.

In calculating results any one of the 10 values which was outside the range of the standard curve or which was 20 per cent above or below the nearest value was discarded. If less than three values were within ± 20 per cent of the average in either duplicate or if the average values for the duplicates did not check within 10 per cent, the determination was always repeated if a sufficient sample was available.

## NICOTINIC ACID (NIACIN)

The procedure of Snell and Wright[13] with the modifications introduced by Krehl, Strong and Elvehjem[14] and some introduced in this laboratory was used for the determination of nicotinic acid. L. arabinosus 17—5, obtained from American Type Culture Collection, Georgetown University Medical School, Washington, D. C., was used as the assay organism.

*Preparation of Standard.*—Nicotinic acid U. S. P., which was kept in a desiccator, was used to prepare the stock standard solution of 100 micrograms per cubic centimeter[15] of distilled water. This solution was kept under benzene in the refrigerator. From this stock standard each week a standard containing 1 microgram of nicotinic acid per cubic centimeter was prepared, and thence standards containing 0.02 and 0.05 microgram per cubic centimeter were prepared daily. Of the standard containing 0.02 microgram per cubic centimeter 1, 2, 3, 4 and 5 cc. were pipetted into five assay tubes, respectively. Two similar series of tubes were prepared with the standard containing 0.05 microgram per cubic centimeter. Thus, the fifteen standard tubes contained amounts of nicotinic acid ranging from 0.02 to 0.25 microgram.

*Preparation of Basal Medium.*—The basal medium contained per hundred cubic centimeters:

| | |
|---|---|
| Casamino acids[16] | 1 Gm. |
| Dextrose | 3 Gm. |
| Sodium acetate | 1.6 Gm. |
| Tryptophan | 20 mg. |
| l-Cystine[17] | 20 mg. |
| l-Asparagine | 40 mg. |
| d-Glutamic acid hydrochloride | 40 mg. |
| Adenine | 2 mg. |
| Guanine | 2 mg. |
| Uracil | 2 mg. |
| Thiamine | 20 micrograms |
| Pyridoxine | 20 micrograms |
| Calcium pantothenate | 20 micrograms |
| Riboflavin | 20 micrograms |
| Paraaminobenzoic acid | 10 micrograms |
| Biotin | 0.1 microgram |
| Salt solution A | 1 cc. |
| Salt solution B | 1 cc. |

The medium was made to volume, adjusted to a $p_H$ of 6.6 to 6.8 with six times normal sodium hydroxide and filtered. The solutions not otherwise noted were prepared according to the method of Krehl and associates.[14] The asparagine and d-glutamic acid hydrochloride, as well as the dextrose and sodium acetate were added in the dry state.

*Preparation of Sample.*—Five cubic centimeter aliquots of milk in duplicate were pipetted into 250 cc. Erlenmeyer flasks. Ninety cubic centimeters of tenth-normal hydrochloric acid was added to each, and the mixtures were autoclaved thirty minutes at 15 pounds (6.8 Kg.) of pressure. After cooling, they were adjusted to $p_H$ 4.6 with six times normal sodium hydroxide to precipitate the casein. After being transferred to 100 cc. volumetric flasks and diluted to volume, with

12. A hole was cut through the top of the photelometer into the curvette carrier to permit placing the culture tubes in the path of light.

13. Snell, E. E., and Wright, L. D.: A Microbiological Method for the Determination of Nicotinic Acid, J. Biol. Chem. **139**:675-686 (June) 1941.

14. Krehl, W. A.; Strong, F. M., and Elvehjem, C. A.: Determination of Nicotinic Acid: Modifications in the Microbiological Method, Indust. & Engin. Chem. (Anal Ed.) **15**:471-475 (July 15) 1943.

15. This standard solution may also be prepared in 50 per cent alcohol and kept in a refrigerator.

16. A preparation, Casamino Acids, obtainable from Difco Laboratories, Detroit, was used in place of the usual casein hydrolysate. A 10 per cent solution of Casamino Acids in distilled water was stirred at room temperature with Darco G 60 at $p_H$ 3 to 3.5 (adjusted with concentrated hydrochloric acid) for thirty to forty minutes. One gram of Darco was used for every 10 Gm. of Casamino Acids. The charcoal was removed by filtration and the solution was stored under benzene in the refrigerator. Casamino Acids varies somewhat between different lots with respect to the amounts of nicotinic acid and biotin present; therefore a second or a third treatment with Darco may be necessary to remove the vitamin contamination in order to obtain good blanks. However, as few treatments with charcoal as are necessary are desirable. Control experiments in which Casamino Acids was compared with casein hydrolysate as used in the original procedure gave similar results.

17. The cystine solution was prepared by dissolving 5 Gm. of l-cystine in a small amount of hot distilled water containing 7 cc. of concentrated hydrochloric acid and diluting to 250 cc. This solution containing 20 mg. per cubic centimeter can be stored at room temperature without a preservative.

xing, they were filtered through no. 42 Whatman er paper. The resulting filtrate, representing a 1:20 ution, was further diluted to a concentration suitable assay—usually 1:50 and 1:80 if the sample was ture milk. Quantities of 1, 2, 3, 4 and 5 cc. of the uted filtrates were added in duplicate to ten assay es, respectively.

Five cubic centimeters of the basal medium was ded to each of the assay tubes (standard tubes and mple tubes prepared as described and two blank tubes ntaining 5 cc. of water). The tubes were autoclaved, culated and incubated, and the growths measured as scribed.

Treatment of milk with enzymes or stronger acid or kali did not significantly affect the values obtained. is confirms the findings of others[18] and indicates t in milk this vitamin is not essentially in a bound te or in the form of a precursor not readily avail- le to L. arabinosus.

*Notes on Determination of Nicotinic Acid.*—A typical ndard turbidity curve obtained with this medium nged from a reading of 71 per cent for the blank to 0 per cent at the 0.25 microgram nicotinic acid level. e titration curve ranged from 1.0 cc. of tenth-normal dium hydroxide for the blank to 6.60 cc. at the same otinic acid level. The titration curve was linear nsiderably beyond 0.25 microgram, but the turbidity

ABLE 1.—*Nicotinic Acid in Two Pooled Samples of Milk Collected Within Ten Days After Parturition and Assayed Repeatedly by the Turbidimetric Method*

| | | Nicotinic Acid | | | |
|---|---|---|---|---|---|
| Sample | Determinations | Range, Micrograms per Cc. | Mean, Micrograms per Cc. | Standard Deviation | Coefficient of Variation |
| ..... | 12 | 3.58-4.33 | 3.82 | 0.193 | 5.05 |
| ..... | 16 | 2.00-2.22 | 2.09 | 0.076 | 3.64 |

rve was not; therefore, the assay was confined to less n this upper limit of concentration. The standard rve varied somewhat from day to day even though the dium and other conditions were kept as constant possible. Despite the day to day variation in the ndard curve, the results when the same sample was n on different days showed good agreement. Two oled samples of milk (I and II) collected within ten ys after parturition were repeatedly assayed turbidi- trically on different days. The results are given in le 1.

No attempt has been made to fractionate the nicotinic id in milk. Whether it is present entirely as the free id or partly as nicotinamide was not determined, as re is no satisfactory way to differentiate between two forms in biologic material. Studies involving ctionation of nicotinic acid and its derivatives in od and urine indicate different metabolic pathways for the free acid and the amide when they are administered in large test doses.[19] Whether this observation holds when normal amounts of nicotinic acid and nicotinamide are obtained from the food is another question.

## PANTOTHENIC ACID

With few exceptions, the basic microbiologic procedure described for the assay of nicotinic acid was employed for the determination of pantothenic acid. L. casei no. 7469 was used as the assay organism.

*Preparation of Standard.*—Pure synthetic d-calcium pantothenate, which had been kept in a desiccator, was used to prepare the stock standard solution of 100 micrograms per cubic centimeter of distilled water. This solution was stored under benzene in the refrigerator. Each week a 1 microgram per cubic centimeter standard was prepared from the stock standard, and from this a standard containing 0.02 microgram per cubic centimeter was prepared daily. From the latter, 0.5, 1, 2, 3, 4 and 5 cc. were pipetted in triplicate into eighteen assay tubes. Thus the dilutions of the standard had a range from 0.01 to 0.1 microgram, which covers the linear part of the turbidity and the titration curve.

*Preparation of Sample.*—Duplicate 5 cc. aliquots of the milk to be assayed were pipetted into 500 cc. Erlenmeyer flasks. To each sample, 175 cc. of distilled water was added, after which the mixtures were autoclaved ten minutes at 15 pounds of pressure. Before cooling, 15 cc. of acetate buffer[20] at $p_H$ 4.6 was added to each flask and the mixtures brought to a boil, if necessary, to precipitate the casein. Each cooled sample was transferred to a 200 cc. volumetric flask, diluted to volume and filtered through no. 42 Whatman paper. The filtrate was diluted to a concentration suitable for assay, usually 1:100 and 1:160 if the sample was mature milk. As in the assay of nicotinic acid, 1 to 5 cc. of each filtrate was pipetted into five assay tubes. Then 5 cc. of basal medium (see "Preparation of Medium") was added to each tube, including the standard tubes just described and two blank tubes each containing 5 cc. of distilled water. The tubes were made up to 10 cc. volume with distilled water, plugged and autoclaved fifteen minutes at 15 pounds of pressure. After cooling and inoculation, the tubes were incubated and the growths measured as described.

Treatment of the milk with enzymes (mylase P or clarase [diastase of malt highly concentrated]) did not significantly affect the pantothenic acid values, indicating that in milk this vitamin is in a free state or at

18. (*a*) Cheldelin, V. H.; Eppright, M. A.; Snell, E., and Guirard, B. M.: Enzymatic Liberation of Vitamins from Plant and Animal Tissues, in Publication 4237, University of Texas, 1942, p. 15-36. (*b*) Cheldelin, V. H., and Williams, R. R.: Extraction and Assay of Nicotinic Acid from Animal and Plant Tissues, Indust. & Engin. Chem. (Anal. Ed.) **14**:671-5 (Aug. 15) 1942. (*c*) Williams, R. J.; Cheldelin, V. H., and Mitchell, H. K.: The B Vitamin Content of Milk from Animals of Different Species, in Publication 4237, University of Texas, 1942, p. 97-104.

19. Handler, P., and Kohn, H. I.: The Mechanism of Cozymase Synthesis in the Human Erythrocyte: A Comparison of the Rôles of Nicotinic Acid and Nicotinamide, J. Biol. Chem. **150**:447-452 (Oct.) 1943. Melnick, D.; Robinson, W. D., and Field, H.: Urinary Excretion of Nicotinic Acid and Its Derivatives by Normal Individuals, ibid. **136**:145-156 (Oct.) 1940. Sarett, H. P.; Huff, J. W., and Perlzweig, W. A.: Studies in Nicotinic Acid Metabolism: I. The Fate of Nicotinic Acid in Man, J. Nutrition **23**:23-34 (Jan.) 1942. Hoagland, C. L.; Ward, S. M., and Shank, R. E.: Synthesis of Coenzyme and Factor V in Erythrocytes, and the Excretion of Nicotinic Acid Derivatives in the Urine Following the Ingestion of Nicotinic Acid and Nicotinamide, J. Biol. Chem. **151**:369-375 (Dec.) 1943.

20. The acetate buffer consisted of 5 Gm. of anhydrous sodium acetate and 3.6 cc. of glacial acetic acid in enough distilled water to make 1 liter.

least in a state in which it is readily utilized by lactobacilli. This confirms the findings of other workers.[21] In most foods and animal tissues a part of the pantothenic acid is bound to the protein and must be freed by autolysis or enzymatic hydrolysis before the microbiologic assay is carried out.

*Preparation of Basal Medium.*—The basal medium employed was similar to that described by Strong, Feeney and Earle,[21a] with the amounts of yeast, dextrose and sodium acetate increased. The basal medium contained per hundred cubic centimeters:

| | |
|---|---|
| Alkali-treated peptone | 1 Gm. |
| Dextrose | 3 Gm. |
| Sodium acetate (supplementary) | 0.4 Gm. |
| Darco-treated yeast supplement | 0.4 Gm. |
| Casamino Acids | 0.4 Gm. |
| l-Cystine | 20 mg. |
| l-Asparagine | 40 mg. |
| d-Glutamic acid hydrochloride | 40 mg. |
| Riboflavin | 20 micrograms |
| Salt solution A | 1 cc. |
| Salt solution B | 1 cc. |

The $p_H$ of the medium was adjusted to 6.6 to 6.8 with sodium hydroxide and the solution filtered before it was added to the assay tubes. The peptone and the yeast were prepared as described by Strong and associates[21a] except that Darco G 60 was used instead of Norit A (a bone charcoal preparation) to treat the yeast (Difco) supplement. The other ingredients were used as in the assay for nicotinic acid.

*Notes on Determination of Pantothenic Acid.*—A typical standard turbidity curve obtained with this medium ranged from a reading of 74 per cent for the blank to 15 per cent at the 0.1 microgram level of calcium pantothenate, whereas the titration curve ranged from 1.5 cc. of tenth-normal sodium hydroxide for the blank to 8.4 cc. at the 0.1 microgram level. A lag in growth frequently was noted at the end of twenty-four hours, which caused the turbidity curve to level off at 19 to 22 per cent. When this occurred readings were repeated after thirty-eight to forty hours' incubation, by which time the maximum growth had been attained. There was considerable variation in both the turbidity and the titration curve from day to day, even though the mediums were prepared in exactly the same way and all other conditions were kept as nearly constant as possible.

TABLE 2.—*Calcium Pantothenate in Two Pooled Samples of Milk Collected Within Ten Days After Parturition and Assayed Repeatedly by the Turbidimetric Method*

| | | Calcium Pantothenate | | | |
|---|---|---|---|---|---|
| Sample | Determinations | Range, Micrograms per Cc. | Mean, Micrograms per Cc. | Standard Deviation | Coefficient of Variation |
| I | 33 | 3.28-4.61 | 3.68 | 0.282 | 7.66 |
| II | 23 | 3.17-3.71 | 3.44 | 0.127 | 3.69 |

The values for any given milk varied somewhat from day to day. If the standard curves showed considerably less growth than average, the values tended to run high. Such assays were repeated.

The same control samples used in the assay for nicotinic acid (table 1) were assayed repeatedly, on different days, for pantothenic acid. The results are given in table 2.

Pantothenic acid can also be determined with L. arabinosus instead of L. casei. With the first organism the medium used in the assay for nicotinic acid[22] or that used in the assay for pantothenic acid may be used.[23] The latter medium, however, was found to give a larger amount of growth for the same concentration of vitamin (corrected for larger blanks), indicating that the former medium is not entirely complete even for L. arabinosus. Numerous samples were assayed simultaneously with L. arabinosus and L. casei, and the values obtained were identical. However, L. arabinosus gave greater growth and acid production than L. casei, which means a higher sensitivity to small amounts of vitamin. This confirmed the findings of Hoag and co-workers.[23] Moreover, the standard curves obtained by using L. arabinosus were more consistently linear in shape. A typical turbidity curve ranged from 69 per cent for the blank to 9 per cent at the 0.1 microgram level of calcium pantothenate. The corresponding titration curve ranged from 3.3 cc. for the blank to 12.5 cc. of tenth-normal sodium hydroxide at the 0.1 microgram level.

## BIOTIN

Biotin was determined microbiologically with the procedure and the basal medium employed in the determination of nicotinic acid, L. arabinosus being used as the assay organism.

*Preparation of Standard.*—The stock standard solution of biotin was prepared from a 50 per cent ethanol solution of crystalline biotin methyl ester containing 25 micrograms per cubic centimeter, obtained from the S. M. A. Corporation. To prepare a stock standard solution containing 0.1 microgram of free biotin per cubic centimeter, 0.84 cc. of the ethanol solution was pipetted, with a specially calibrated pipet, into a 200 cc. volumetric flask. Ten cubic centimeters of five tenths-normal sodium hydroxide was added to the biotin methyl ester solution and allowed to stand thirty minutes to hydrolyze the ester. The mixture was then neutralized with dilute hydrochloric acid to approximately $p_H$ 7.0, diluted to volume (200 cc.) and stored in the refrigerator under benzene.[24] A standard solution containing 1 millimicrogram per cubic centimeter was prepared from the stock standard solution. The dilute standard (1 millimicrogram per cubic centimeter) was prepared weekly and stored under benzene in the refrigerator. Standard solutions containing 0.05 and 0.1 millimicrogram per cubic centimeter were prepared daily from this dilute standard. Of the 0.05 millimicrogram standard 1, 2, 3, 4 and 5 cc. were pipetted into five assay tubes, and similar amounts of the 0.2 millimicrogram standard were pipetted in duplicate into ten assay tubes. Thus, the dilutions of the standard had a range of 0.05 to 1 millimicrogram of biotin.

21. (*a*) Strong, F. M.; Feeney, R. E., and Earle, A.: Microbiological Assay for Pantothenic Acid, Indust. & Engin. Chem. (Anal. Ed.) **13**:566-570 (Aug. 15) 1941. (*b*) Williams, Cheldelin and Mitchell.[18c]

22. Skeggs, H. R., and Wright, L. D.: The Use of Lactobacillus Arabinosus in the Microbiological Determination of Pantothenic Acid, J. Biol. Chem. **156**:21-26 (Nov.) 1944.

23. Hoag, E. H.; Sarett, H. P., and Cheldelin, V. H.: Use of Lactobacillus Arabinosus 17-5 for Microassay of Pantothenic Acid, Indust. & Engin. Chem. (Anal. Ed.) **17**:60-62 (Jan.) 1945.

24. The biotin solution has also been prepared from crystalline free acid biotin more recently supplied by the S. M. A. Corporation. With this preparation, the alkaline hydrolysis is unnecessary.

*Preparation of Sample.*—The same diluted sample filtrates of milk used in the assay for nicotinic acid were used for the determination of biotin. The procedure was carried out exactly as for nicotinic acid.

Treatment of the milk with water or with stronger acid gave essentially the same values as did treatment with tenth-normal hydrochloric acid, which is in agreement with the observations of other workers.[25] Treatment with enzymes (papain, clarase, mylase P) was unsatisfactory, since the biotin content of the enzymes was large in comparison with that of the milk. No significant increase in biotin values was noted after enzyme treatment, in contrast to the findings of Cheldelin and co-workers.[18a] In assaying colostrum, the water-autoclaved filtrates prepared for the determination of pantothenic acid were used instead of the acid filtrates because the latter were deep yellow and seemed to contain more interfering substances than the water-extracted milk filtrates. However, the biotin levels in early milks were so low that few reliable values were obtained with either type of extraction. The turbidity and titration values of these samples, moreover, often differed greatly, indicating the presence of considerable amounts of interfering substances.

*Preparation of Basal Medium.*—The same medium was used as in the determination of nicotinic acid except that biotin was omitted from the medium and 10 micrograms of nicotinic acid per hundred cubic centimeters substituted.

*Notes on Determination of Biotin.*—A typical standard turbidity curve ranged from a reading of 70 per cent for the blank to a reading of 10 per cent for 1 millimicrogram of biotin per cubic centimeter. The titration curve ranged from 2 cc. of tenth-normal sodium hydroxide for the blank to 13 cc. at the 1 millimicrogram level. The standard curves may vary considerably from day to day. These variations may be accounted for partially by possible differences in the amounts of biotin carried over in the drop of inoculum or variations in the traces of biotin remaining in the charcoal-treated Casamino Acids solutions. However, most of the variations are caused by unidentified and hence uncontrollable factors.

The same samples assayed for nicotinic acid (table 1) were repeatedly assayed, on different days, for biotin. The results are given in table 3.

TABLE 3.—*Biotin in Two Pooled Samples of Milk Collected Within Ten Days After Parturition and Assayed Repeatedly by the Turbidimetric Method*

| Sample | Biotin | | | | |
|---|---|---|---|---|---|
| | Determinations | Range, Millimicrograms per Cc. | Mean, Millimicrograms per Cc. | Standard Deviation | Coefficient of Variation |
| I | 12 | 10.2-12.8 | 11.38 | 0.817 | 7.18 |
| II | 14 | 7.60-9.08 | 8.24 | 0.406 | 4.93 |

## RESULTS AND COMMENTS

*Nicotinic Acid.*—Chart 1 shows the daily variation in the nicotinic acid content of human milk collected over twenty-four hour periods during the first ten days following parturition from mothers who were on comparable known diets. On the first day the mean nicotinic acid content of the colostrum of 5 women was 100 micrograms per hundred cubic centimeters. The means of the daily values decrease on the second and third days but increased after the fourth day, reaching 245 micrograms on the tenth day. Although the percentage increase is not quite as great, the rise in the nicotinic acid values during

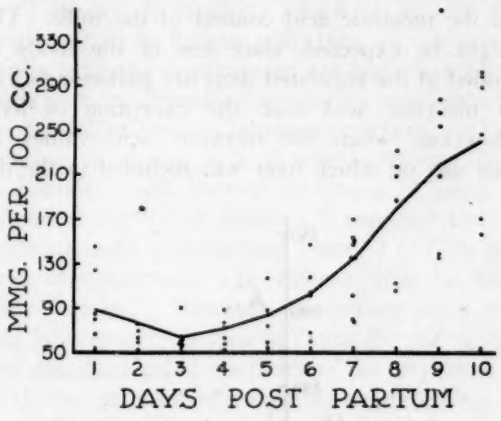



Chart 1.—Nicotinic acid in twenty-four hour collections of immature human milk. The curve was obtained by three point smoothing of the means. In this and subsequent charts the term "microgram" (one thousandth of a milligram) is abbreviated to mmg.

the first ten days resembles that in the thiamine values,[26] since the concentrations of both vitamins increase steadily from comparatively low values on the third day to values several times higher on the tenth day.

The nicotinic acid values obtained for mature milk of both the group of women on known diets and the group on self-chosen diets are shown in chart 2. Although the best-fitting curve for the group of women receiving known diets (*AA*) decreases slightly as lactation progresses, this is of little significance, for the small number of determinations obtained during the later months of lactation unduly influence the calculated curve. The values for the four to eight hour samples from women eating self-chosen diets show much more even "scatter" throughout the first ten months of lactation, and the almost horizontal curve at approximately 180 micrograms per hundred cubic centimeters accurately represents the nicotinic acid content of mature milk after the first postpartum month. Whether or not the average nicotinic acid content of the milk continues to increase after the tenth day,

25. Lampen, J. O.; Babler, G. P., and Peterson, W. J.: The Occurrence of Free and Bound Biotin, J. Nutrition **23**:11-21 (Jan.) 1942. Cheldelin and others.[18a]

26. Roderuck, C. E.; Williams, H. H., and Macy, I. G.: Human Milk Studies: XXIII. Free and Total Thiamine Contents of Colostrum and Mature Human Milk, Am. J. Dis. Child., this issue, p. 162.

and if so how much, is not evident from the present data. There are too few values on milks collected between ten and forty days post partum for one to make any definite statements. In table 4 the ranges and the mean values, with the standard deviations, are given for the two groups of women.

The daily variations in nicotinic acid content show no parallelism between individual subjects, which would indicate that the diet has little effect on the nicotinic acid content of the milk. This might be expected, since few of the foods included in the regulated diets are particularly rich in nicotinic acid with the exception of liver. However, when the nicotinic acid values for that day on which liver was included in the diet were compared, it was noted that in 7 cases they increased, in 7 they decreased and in 3 they remained stationary. This lack of response to liver in the diet is rather surprising, but the same is true of the milk of women on self-chosen diets; no relationship was found between the nicotinic acid content of the milk and the liver or any other good source of nicotinic acid in the diet. Moreover, no relationship between the concentration of nicotinic acid and the time after parturition, the season of the year or the volume of milk produced could be found. This lack of response to the diet is explainable, however, when the nicotinic acid of the blood is considered, since the constituents of the milk must come directly or indirectly from the blood. In a study of factors affecting the concentration and distribution of nicotinic acid in the blood, Melnick, Robinson and Field [27] demonstrated that the nicotinic acid level in whole blood is unaffected by fasting or recent ingestion of a meal. Klein,

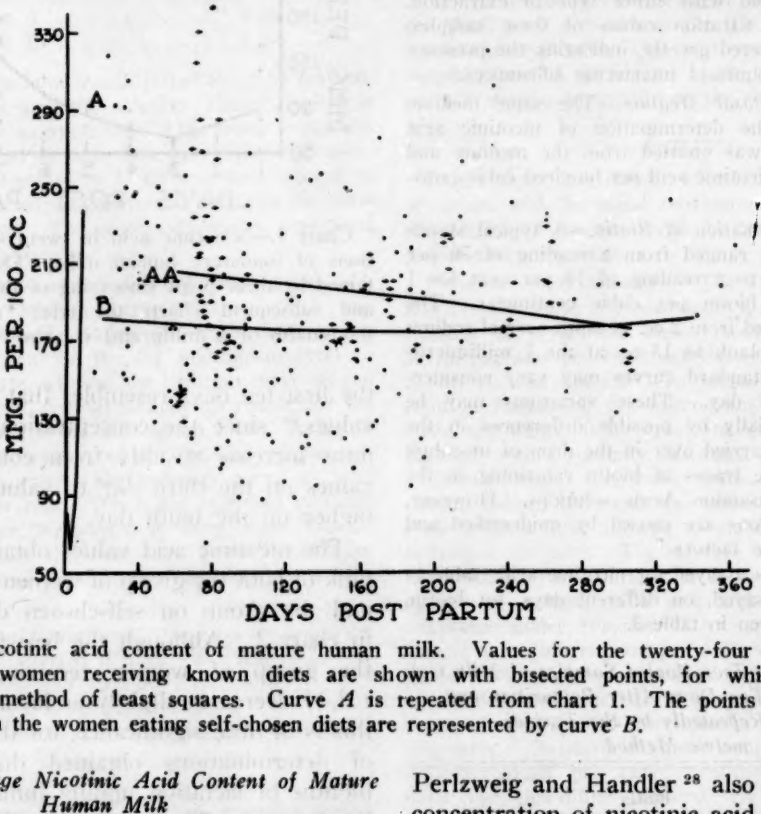



Chart 2.—Nicotinic acid content of mature human milk. Values for the twenty-four hour collections of milk from the women receiving known diets are shown with bisected points, for which curve *AA* was obtained by the method of least squares. Curve *A* is repeated from chart 1. The points showing values for the samples from the women eating self-chosen diets are represented by curve *B*.

TABLE 4.—*Average Nicotinic Acid Content of Mature Human Milk*

| | | Nicotinic Acid | | |
|---|---|---|---|---|
| Group | Samples | Range, Micrograms per 100 Cc. | Mean, Micrograms per 100 Cc. | Standard Deviation |
| Women on known diets..... | 86 | 128-330 | 196 | 38.2 |
| Women on self-chosen diets. | 182 | 66-320 | 176 | 51.3 |
| Total..... | 268 | 66-330 | 183 | 48.4 |

Perlzweig and Handler [28] also observed that the concentration of nicotinic acid in the blood corpuscles was not affected appreciably by the dietary state. Approximately 90 per cent of the blood content of nicotinic acid is in the cells.

Possibly another factor, intestinal bacteria, may prove to be of considerable importance in the problem of the nicotinic acid supply of the

27. Melnick, D.; Robinson, W. D., and Field, H. Factors Affecting the Concentration and Distribution of Nicotinic Acid in the Blood, J. Biol. Chem. **136**:15 166 (Oct.) 1940.

28. Klein, J. R.; Perlzweig, W. A., and Handler, P. Determination of Nicotinic Acid in Blood Cells and Plasma, J. Biol. Chem. **145**:27-34 (Sept.) 1942.

body. Recently, Ellinger and associates[29] have obtained experimental data which would indicate that the quantity of nicotinic acid provided for human utilization by the intestinal flora may amount to as much as 80 per cent of the daily intake. If true, this would mask any affects from common variations in diet.

A series of studies on the nicotinic acid in human milk were made by Lwoff and co-workers[30] with a microbiologic method in which the growth of Bacterium proteus is measured turbidimetrically.[31] The fact that most of the milk samples were single collections made at 11 a. m., before the nursing, and, further, that the diets were not uniform may account in part for the considerable variation seen in their results. The authors stated, however, that about 100 Gm. of meat was eaten daily by each woman. Starting with the second to sixth postpartum day, daily samples from 8 women were assayed on five to eleven of the following days. The milk of 6 of these women showed a small initial drop to values of 50 to 90 micrograms per hundred cubic centimeters, followed by a marked increase to values of 130 to 340 micrograms by the ninth to the thirteenth day, averaging 180 micrograms on the tenth day. In general, their results are very similar to those of the present study. The milk of 1 woman in Lwoff's group was assayed through the twenty-first day. The level of nicotinic acid fell slowly but steadily after the thirteenth day, when it was 290 micrograms, to 190 micrograms on the last day. The amount in the milk of another woman decreased from a maximum of 320 micrograms on the sixteenth day to 170 micrograms on the twenty-third day, after which it remained quite constant throughout the thirtieth day. These workers also made twenty-four determinations on single samples of milk collected between fifty-one and four hundred and twenty-five days post partum and obtained values ranging from 80 to 250 micrograms, with an average of 150 micrograms per hundred cubic centimeters.

The nicotinic acid contents of 9 milk samples from 5 women three to twenty days after parturition were determined microbiologically by Williams, Cheldelin and Mitchell.[18c] The average value was 160 micrograms per hundred cubic centimeters; however, there was no definite increase in the values with increase in days after parturition—the values ranged only from 120 to 220 micrograms. In comparing human milk with the milk of the mare, the cow, the goat, the dog and the mouse in vitamin content, Williams and his group have shown that nicotinic acid is unique in that it is the only one of the more well known B vitamins which is present at a higher level in human milk than in cow's milk. Folic acid and inositol also are higher in human milk but, in general, the vitamin concentrations in the milks tend to increase progressively from human to mouse milk in the foregoing order.

Escudero and Pertusi de Esquef,[32] using the chemical method of Kodicek,[33] reported that the nicotinic acid in colostrum (second to fifth day) was unmeasurable. In mature milk he found only "traces." However, in another paper from his laboratory,[34] values of 60 to 80 micrograms per hundred cubic centimeters are reported for 4 pooled samples of colostrum representing the second to fourth day of lactation, from normal women on fair diets. These values are very similar to those found in this laboratory at that period of lactation (chart 1).

Kodicek,[33] using a chemical method and water extraction, reported the nicotinic acid in the milk of 3 women merely as less than 100 micrograms per hundred cubic centimeters in every sample. No information regarding the period of lactation was given. Teply, Strong and Elvehjem,[35] using the microbiologic procedure of Snell and Wright and water extraction, reported a value of 0.26 milligram per hundred cubic centimeters for human milk. No information was given concerning the sample used, however. This difference may be attributable in part at least to the differences in the assay methods employed. In cow's milk, for which many more values have been reported, the microbiologic assays by different workers give average values between 60 and

29. Ellinger, P.; Coulson, R. A., and Benesch, R.: Production and Release of Nicotinamide by the Intestinal Flora in Man, Nature, London **154**:270-271 (Aug. 26) 1944.

30. Lwoff, A., and Morel, M.: L'évolution de la teneur du lait de la femme en nicotinamide, Compt. rend. Soc. de biol. **136**:187-192, 1942.

31. Lwoff, A., and Querido, A.: Dosage de l'amide de l'acide nicotinique au moyen du test proteus: Principe de la methode, Compt. rend. Soc. de biol. **129**: 1039-1043, 1938.

32. Escudero, P., and Pertusi de Esquef, L.: Estudios sobre el calostro humano: La alimentación del niño en los primeros cinco días de su vida, Rev. Asoc. argent. de dietol. **2**:107-113 (April-June) 1944.

33. Kodicek, E.: Estimation of Nicotinic Acid in Animal Tissues, Blood and Certain Foodstuffs: I. Method, Biochem. J. **34**:712-723, 1940; Estimation of Nicotinic Acid in Animal Tissues, Blood and Certain Foodstuffs: II. Applications, ibid. **34**:724-735, 1940.

34. Herraiz, M. L., and Herrero, H. G.: Contenido de vitaminas del calostro humano, Rev. Asoc. argent. de dietol. **1**:95-98 (April-June) 1943.

35. Teply, L. J.; Strong, F. M., and Elvehjem, C. A.: The Distribution of Nicotinic Acid in Foods, J. Nutrition **23**:417-423 (April) 1942.

100 micrograms per hundred cubic centimeters,[36] whereas chemical assays by various workers have given values ranging from 60 to 820 micrograms per hundred cubic centimeters.[37]

*Pantothenic Acid.*—The daily variation in the calcium pantothenate content of the immature milk obtained during the first ten days following parturition is shown in chart 3. As with the other B vitamins, pantothenic acid is at a relatively low level on the first day, the calcium pantothenate averaging 48 micrograms per hundred cubic centimeters. Unlike thiamine and nicotinic acid, pantothenic acid rises abruptly during the first three or four days. By the fourth day the mean concentration of the calcium pantothenate is 245 micrograms per hundred cubic centimeters, after which the increase is slight through the tenth day, when the mean is 304 micrograms. Since samples were not collected from the women on known diets between the tenth day and the second month post partum, and since rather few samples were obtained from the women on self-chosen diets in this interval, it is not known whether or not the concentration of panthothenic acid in the milk continues to increase after the tenth day. It apparently decreases somewhat, however, between the tenth day and the second month post partum.

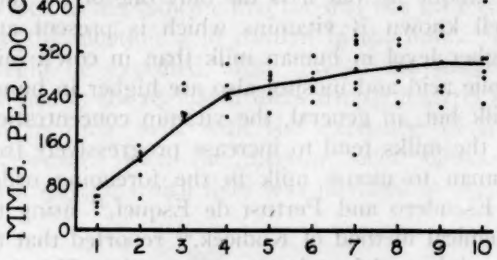



Chart 3.—Calcium pantothenate in twenty-four hour collections of immature human milk. The curve was obtained by three point smoothing of the means.

Nine samples of milk obtained from 5 apparently normal women three to twenty days after parturition were assayed by Williams and coworkers [18c] and found to contain from 77 to 300 micrograms of calcium pantothenate per hundred cubic centimeters with an average of 160 micrograms. Comparison of human milk with the milk of various animals showed that the mean pantothenic acid values for human milk were lower than those for the milk of the mare, the cow, the goat, the dog or the mouse. However,

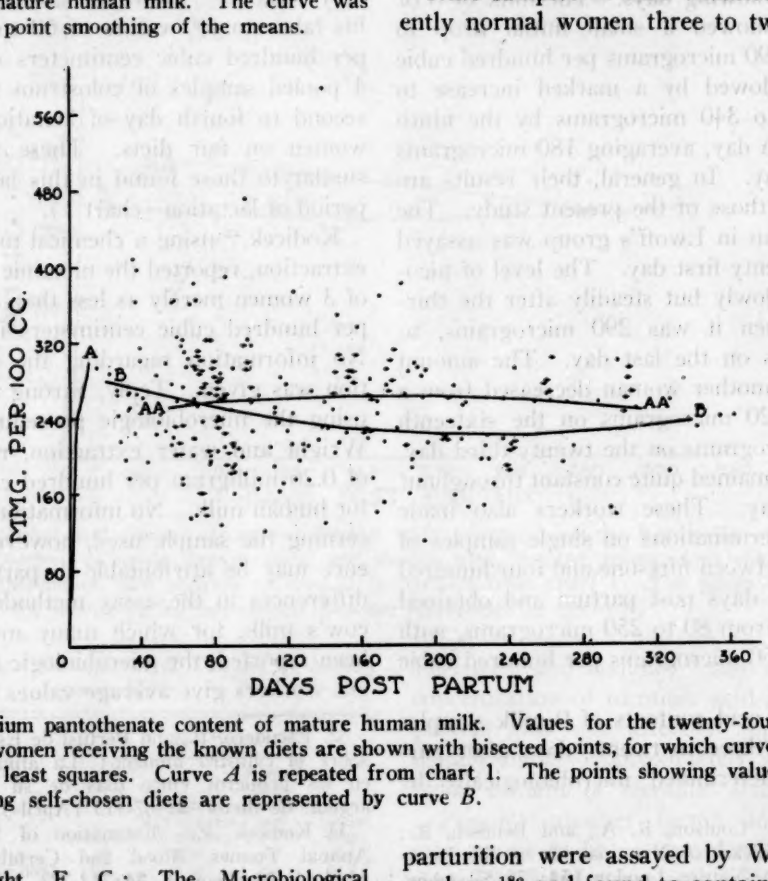



Chart 4.—Calcium pantothenate content of mature human milk. Values for the twenty-four hour collections of milk from the women receiving the known diets are shown with bisected points, for which curve *AA* was obtained by the method of least squares. Curve *A* is repeated from chart 1. The points showing values for the samples from women eating self-chosen diets are represented by curve *B*.

36. Barton-Wright, E. C.: The Microbiological Assay of Nicotinic Acid in Cereals and Other Products, Biochem. J. **38**:314-319, 1944. Williams and others.[18c]

37. Noll, C. I., and Jensen, O. G.: The Chemical Determination of Nicotinic Acid in Milk and Milk Derivatives, J. Biol. Chem. **140**:755-762 (Sept.) 1941. Waisman, H. A., and Elvehjem, C. A.: Chemical Estimation of Nicotinic Acid and Vitamin $B_6$, Indust. & Engin. Chem. (Anal. Ed.) **13**:221-225 (April 15) 1941.

except for the cow, only 1 to 4 samples of the milk of each animal were assayed. The authors stated that in human milk the pantothenic acid appears to diminish slightly during lactation. However, the samples are too few for reliable conclusions.

The calcium pantothenate values for mature milk from the women receiving known diets and the women eating self-chosen diets are shown in chart 4. For both groups, the best

TABLE 5.—*Average Calcium Pantothenate Content of Mature Human Milk*

| Group | Samples | Calcium Pantothenate: Range, Micrograms per 100 Cc. | Calcium Pantothenate: Mean, Micrograms per 100 Cc. | Calcium Pantothenate: Standard Deviation |
|---|---|---|---|---|
| Women on known diet..... | 86 | 137-347 | 256 | 44.3 |
| Women on self-chosen diets. | 183 | 86-584 | 242 | 69.9 |
| Total..... | 269 | 86-584 | 246 | 63.1 |

fitting curve for the mean daily secretion of pantothenic acid shows little difference between the mean value ten days post partum and that from one to two months later. As lactation progressed, the mean amounts in the milk from both groups of women remained relatively constant and, in general, the values show little scatter.

In table 5 the ranges and the mean values, with their standard deviations, are given for the two groups of women. Since few common foods contain unusually high amounts of pantothenic acid, little correlation between special foods in the self-chosen diets and high pantothenate contents of milk could be found. However, although relatively small, the variations from day to day are fairly consistent among the women on regulated diets. For example, in 17 five day studies, 12 of the 17 collections on the third day showed increases over the previous day and 13 of those on the fifth day showed increases over the fourth day. The consistent increase on the last day is undoubtedly due to the presence of liver in the diet. Liver is a good source of pantothenic acid.

*Biotin.*—The biotin content of immature milk during the first ten days following parturition is shown in chart 5. The levels of biotin for the first four days are so low that they cannot be determined with any degree of reliability. There are probably traces of biotin present, but it can be recorded only as less than 0.10 microgram per hundred cubic centimeters, and in most cases is probably less than 0.06 microgram. The mean value on the fifth day is 0.13 microgram per hundred cubic centimeters. It rises slowly to a value of 0.38 microgram on the ninth day, which is less than half the average value of 0.80 microgram per hundred cubic centimeters for the mature milk of the women on regulated diets at two to three months post partum. Apparently, biotin continues to increase after the ninth day until it reaches the mature value some time before two to three months. Whether it may surpass the mature value and then decrease again in this interim is not determinable from the present data.

On the tenth day, liver, a good source of biotin, was included in the diet, which greatly influenced the biotin level in the milk for that day. Although the range of the values was great, 0.09 to 2.56 micrograms per hundred cubic centimeters, the average, 1.22 micrograms, was nearly four times that found for the ninth day. It is this very high value on the tenth day which causes the smoothed mean curve (shown in chart 5) to rise abruptly after the eighth day. It is interesting that although liver was also included in the diet on the fifth day, no significant effect on the biotin values was apparent. This lack of response to liver is probably connected with the original very low level of biotin in the milk. This may indicate that the mammary tissue is unable to secrete true biotin by the fifth day and that the "traces" found before that time might be due to interfering factors or to biotin vitamers.

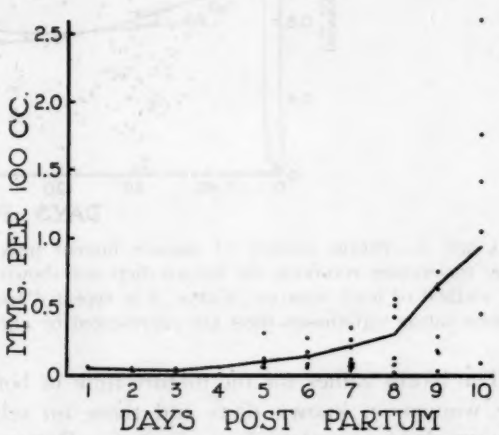



Chart 5.—Biotin in twenty-four hour collections of immature human milk. The curve was obtained by three point smoothing of the means.

The biotin contents of 8 milk samples from 4 women three to twenty days after parturition were determined microbiologically by Williams and co-workers,[18c] who used Saccharomyces cerevisiae as the test organism. They recorded less than 0.2 microgram per hundred cubic centimeters for the milk samples collected three to eight days after parturition. For 1 sample ob-

tained at ten days and 1 at twenty days the values 0.5 and 1.8 microgram per hundred cubic centimeters, respectively, were recorded. However, they stated that these 2 samples were obtained from women who had been receiving daily vitamin supplements during their pregnancy, which may account for the higher values.

TABLE 6.—*Average Biotin Content of Mature Human Milk*

| | | Biotin | | |
|---|---|---|---|---|
| Group | Samples | Range, Micrograms per 100 Cc. | Mean, Micrograms per 100 Cc. | Standard Deviation |
| Women on known diet..... | 85 | 0.039-2.066 | 0.80 | 0.484 |
| Women on self-chosen diets.. | 181 | 0.104-4.220 | 0.82 | 0.490 |
| Total..... | 266 | 0.039-4.220 | 0.81 | 0.488 |

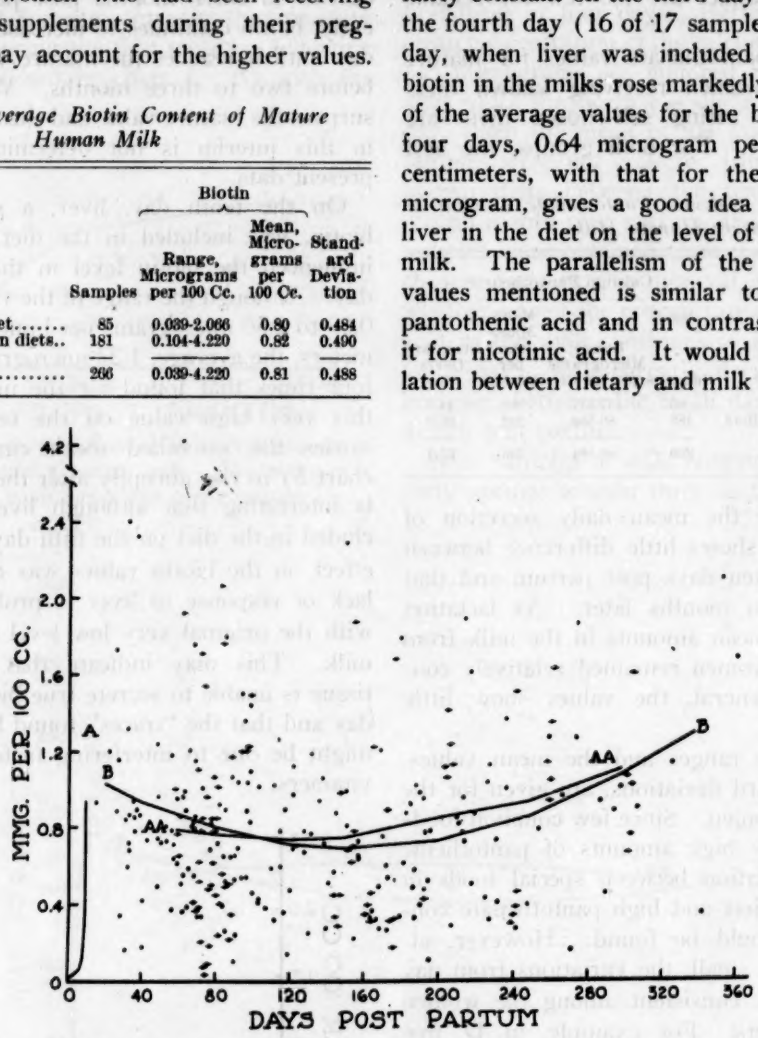



Chart 6.—Biotin content of mature human milk. Values for the twenty-four hour collections of milk from the women receiving the known diets are shown with bisected points, for which curve *AA* was obtained by the method of least squares. Curve *A* is repeated from chart 1. The points showing values for samples from the women eating self-chosen diets are represented by curve *B*.

The biotin values for the mature milk of both the women on known diets and those on self-chosen diets are plotted in chart 6. Between the second and sixth months of lactation the amounts in the milk of both groups of women are relatively constant, 0.7 to 0.8 microgram per hundred cubic centimeters. The rise in the curves after the sixth month probably is attributable to the smaller number of values obtained during the later months of lactation. The mean values, the ranges and the standard deviations for the two groups of women are given in table 6.

When the individual daily variations of the biotin content of the milk of the women on the regulated diet were compared, it was noted that, though small, they were rather similar. The biotin decreased slightly on the second day as compared with the first (13 of 17 samples), was fairly constant on the third day but increased on the fourth day (16 of 17 samples). On the fifth day, when liver was included in the diet, the biotin in the milks rose markedly. A comparison of the average values for the biotin of the first four days, 0.64 microgram per hundred cubic centimeters, with that for the fifth day, 1.46 microgram, gives a good idea of the effect of liver in the diet on the level of biotin in mature milk. The parallelism of the individual daily values mentioned is similar to that noted for pantothenic acid and in contrast to the lack of it for nicotinic acid. It would indicate a correlation between dietary and milk biotin.

No relationship between the concentration of biotin in mature milk and the time after parturition or the volume of milk produced could be found.

## SUMMARY

Nicotinic acid, pantothenic acid and biotin were determined in human milk secreted during the first ten days post partum and in mature milk obtained as late as the twelfth month of lactation. In the assays for these vitamins microbiologic procedures were employed.

The mean nicotinic acid content of the milk decreased slightly in the first two to four days

ter parturition and then rose rather rapidly. n the tenth day the mean value was 245 micro-rams per hundred cubic centimeters. In twenty-our hour collections of mature human milk from omen on known diets, planned by a dietitian, e average nicotinic acid content was 196 micro-rams per hundred cubic centimeters, and in our to eight hour collections from women on lf-chosen diets the average was 176 micrograms er hundred cubic centimeters.

The average pantothenic acid content of the ilk increased rather rapidly, the calcium panto-enate rising from 48 micrograms per hundred ubic centimeters on the first day post partum to 45 micrograms on the fourth day. From the ourth to the tenth day the increase was gradual. he average for the tenth day was 304 micro-rams per hundred cubic centimeters. The mean alcium pantothenate content of mature human ilk is 256 micrograms for the women on known iets and 242 micrograms for the women on self-osen diets.

The biotin content of the milk remained extremely low for the first four or five days after parturition; at the end of that time it started to increase gradually, reaching an average value of 0.38 microgram per hundred cubic centimeters on the ninth day. The mean biotin content of mature human milk was 0.80 microgram for the women on known diets and 0.82 microgram for the women on self-chosen diets.

No effect of duration of lactation, volume of milk or season of the year could be noted on the values of any of these three vitamins in human milk.

NOTE.—Since the manuscript for this paper was submitted for publication, we have received copies of three manuscripts from Dr. L. A. Maynard, of Cornell University, which contain data for cow's milk comparable to those given in this paper for human milk (Lawrence, J. M.; Herrington, B. L., and Maynard, L. A.: Some B Vitamins in Cow's Milk: I. Nicotinic Acid, *J. Nutrition*, to be published; II. Biotin, ibid., to be published; III. Pantothenic Acid, ibid., to be published).

# HUMAN MILK STUDIES

## XXIII. FREE AND TOTAL THIAMINE CONTENTS OF COLOSTRUM AND MATURE HUMAN MILK

CHARLOTTE E. RODERUCK, M.S.; HAROLD H. WILLIAMS, PH.D., AND ICIE G. MACY, PH.D.

DETROIT

The original data on normal human milk obtained by Macy and associates [1] include the first quantitative measurements of vitamin B, now known as thiamine, in human milk. There had been several earliers reports concerning the fact that the quality of milk produced by mothers whose babies had died of beriberi was inferior. In 1912 Andrews [2] reported that 16 puppies fed milk from such mothers exhibited incoordination and weakness of extremities within three or four weeks, finally dying. He concluded:

> . . . in the Philippines the mortality is greatest among breast-fed children, possibly because of the poor quality of mother's milk. The latter is probably deleterious by reason of what it lacks rather than because of any harmful constituent. It seems probable that there is an intimate relation between beriberi of infants and a mother's milk poor in quality and lacking certain necessary elements which are not included in the mother's dietary.

Gibson and Concepción [3] found that 20 cc. of human milk did not prevent birds on a polished rice diet from having polyneuritis. Since Vedder and Clark [4] had found that 5 cc. of fresh cow's milk did prevent polyneuritis under the same conditions, Gibson [5] stated that "normal human milk must contain not more than one-fourth the amount of vitamin of the former." Subnormal growth of rats was noted by Osborne and Mendel [6] when 5 cc. of human milk was fed daily as the only source of vitamin B in a ration otherwise adequate. Only fairly good growth resulted when desiccated milk was fed in amounts equivalent to 10 cc. of whole milk daily. Kennedy, Palmer and Schlutz,[7] in 1922, demonstrated that 10 cc. of breast milk given daily as the sole source of vitamin B was inadequate for normal growth of the rat.

Thiamine is directly concerned with the metabolism of carbohydrates,[8] and Richter and Rice [9] recently presented evidence that it also affects the metabolism of proteins. Reinhold, Nicholson and Elsom [10] have shown that fat has no thiamine-sparing action as believed formerly. The high fat diets were always accompanied by low intake of carbohydrate and thus the thiamine required for utilization of the carbohydrate was reduced. That there is a correlation between the dietary intake of thiamine and the concentration of thiamine in breast milk has been suggested by most investigators. The refined and limited diets of the present time frequently provide insufficient intakes of thiamine for optimal health. Moderate but prolonged restriction of thiamine has been shown to produce emotional

From the Research Laboratory, Children's Fund of Michigan.

1. (*a*) Macy, I. G.; Outhouse, J.; Graham, A., and Lang, M. L.: Human Milk Studies: III. The Quantitative Estimation of Vitamin B, J. Biol. Chem. **73**:189-201, 1927. (*b*) Donelson, E. G., and Macy, I. G.: Human Milk Studies: XII. The Vitamin B and Vitamin G Content Before and During Maternal Consumption of Yeast, J. Nutrition **7**:231-249 (Feb.) 1934.

2. Andrews, V. L.: Infantile Beriberi, Philippine J. Sc. **7**:67-89 (April) 1912.

3. Gibson, R. B., and Concepción, I.: The Influence of Fresh and Autoclaved Cow's Milk on the Development of Neuritis in Animals, Philippine J. Sc. **11**:119-131, 1916.

4. Vedder, E. B., and Clark, E.: A Study of Polyneuritis Gallinarium, Philippine J. Sc. **7**:423-461 (Oct.) 1912.

5. Gibson, R. B.: The Protective Power of Normal Human Milk Against Polyneuritis Gallinarium (Beriberi), Philippine J. Sc. **8**:469-473 (Dec.) 1913.

6. Osborne, T. B., and Mendel, L. B.: Milk as a Source of Water-Soluble Vitamin III, Biochem. J. **16**: 363-367, 1922.

7. Kennedy, C.; Palmer, L. S., and Schlutz, F. W.: The Vitamin Content of Breast Milk, Tr. Am. Pediat. Soc. **35**:26-33, 1923.

8. Boxer, G. E., and Stetten, DeW.: Role of Thiamine in Synthesis of Fatty Acids from Carbohydrate Precursors, J. Biol. Chem. **153**:607-616 (May) 1944. Chesler, A.; Homburger, E., and Himwich, H. E.: Carbohydrate Metabolism in Vitamin $B_1$ Deficiency, ibid. **153**:219-225 (April) 1944.

9. Richter, C. P., and Rice, K. K.: Comparison of the Nutritive Value of Dextrose and Casein and of the Effects Produced on Their Utilization by Thiamine, Am. J. Physiol. **141**:346-353 (May) 1944.

10. Reinhold, J. G.; Nicholson, J. T. L., and Elsom, K. O.: The Utilization of Thiamine in the Human Subject: The Effect of High Intake of Carbohydrate or of Fat, J. Nutrition **28**:51-62 (July) 1944.

instability, reflected by irritability, moodiness, lack of cooperation and mental depression.[11]

One of Macy's early studies [12] showed that the lactating rat had a thiamine requirement three to five times greater than that of the normal adult rat. This observation was confirmed by Evans and Burr [13] and by Sure.[14] Slater and Rial [15] pointed out that during lactation rats increase their food consumption, and therefore their thiamine requirement, two to three times. However, human beings augment their food intake by only about 20 per cent during lactation; so their thiamine requirements might not be increased by more than the same percentage. Utheim Toverud [16] found that the pregnant woman requires four to five times as much thiamine as the normal, nonpregnant women but that the lactating woman's requirement lies between these two. Lockhart, Kirkwood and Harris [17] found the thiamine requirement of lactating women to be three times that of nonpregnant women. Morgan and Haynes,[18] studying 2 women, one with an excellent dietary background, the other with a poor background, demonstrated that the thiamine concentration in the milk of the latter could be improved but not that in the milk of the woman whose previous dietary was excellent. Thus, an effect of diet was indicated as well as a physiologic limitation of the mammary gland. Slater and Rial [15] have confirmed this conception. In 1932 Gunderson and Steenbock [19] found evidence of physiologic control of the vitamin B content of both cow's and goat's milk.

Increased physiologic activity, such as that in growth or lactation, necessitates an increased intake of thiamine, and investigations of human milk provide information which can be used in estimating thiamine requirements. The studies of infant growth made in this laboratory,[20] while they did not show increased rates for infants receiving as a supplement an extract of rice polishings, did indicate that the growth of the infants was more consistent. This has been designated as a "stabilizing" effect of thiamine. Colby and co-workers,[21] in psychologic studies of the same children, observed that at 6 months visual pursuit, sustained fixation, eye-hand coordination patterns and prehension were definitely superior in the group receiving additional vitamin B.

In an extensive, well controlled experiment involving subjects 4 to 20 years of age, Harrell [22] established the superiority of a group receiving 2 mg. of thiamine daily in addition to their diet over a group receiving only the regular diet, which supplied an average of 0.9 mg. of thiamine per day. This superiority was apparent in eighteen activities. It was statistically significant at the 1 per cent level in code learning and "underlining 4's" and at the 5 per cent level in "reading speed" and accuracy, division, number span, left-handed grip and addition. In other activities the difference was not statistically significant but was consistently better.

As early as 1928, Hoobler [23] described symptoms of vitamin B deficiency in infants in America. These included loss of appetite, failure to gain weight, marked nervous disabilities, such as stiffness of the neck, the arms and the legs, and profound degenerative changes in the digestive system. All of these quickly disappeared when yeast or an extract of rice polishings was added to the diet. In 1931, reporting on 125 babies in a hospital nursery in Detroit, Hoobler [24] stated that "about 1 out of 6 of the infants studied

11. Williams, R. D.; Mason, H. L.; Smith, B. F., and Wilder, R. M.: Induced Thiamine Deficiency and the Thiamine Requirement of Man, Arch. Int. Med. **69**: 721-738 (May) 1942.

12. Macy, I. G.; Outhouse, J.; Long, M. L., and Graham, A.: Human Milk Studies: I. Technique Employed in Vitamin Studies, J. Biol. Chem. **73**:153-174 (May) 1927.

13. Evans, H. M., and Burr, G. O.: On the Amount of Vitamin B Required During Lactation, J. Biol. Chem. **76**:263-272 (Jan.) 1928.

14. Sure, B.: Quantitative Requirements of the Components of the Vitamin B Complex for Lactation and Growth of Nursing Young of the Albino Rat, J. Nutrition **19**:57-69 (Jan.) 1940.

15. Slater, E. C., and Rial, E. J.: Thiamin (Vitamin $B_1$) Content of Human Milk, M. J. Australia **1**:3-12 (Jan. 3) 1942.

16. Utheim Toverud, K.: The Excretion of Aneurin in Pregnant and Lactating Women and in Infants, Ztschr. f. Vitaminforsch. **10**:255-267, 1940.

17. Lockhart, H. S.; Kirkwood, S., and Harris, R. S.: The Effect of Pregnancy and Puerperium on the Thiamine Status of Women, Am. J. Obst. & Gynec. **46**:358-365 (Sept.) 1943.

18. Morgan, A. F., and Haynes, E. G.: Vitamin $B_1$ Content of Human Milk as Affected by Ingestion of Thiamin Chloride, J. Nutrition **18**:105-114 (Aug.) 1939.

19. Gunderson, F. L., and Steenbock, H.: Is the Vitamin B Content of Milk Under Physiological Control? J. Nutrition **5**:199-212 (March) 1932.

20. Poole, M. W.; Hamil, B. M.; Cooley, T. B., and Macy, I. G.: Stabilizing Effect of Increased Vitamin B ($B_1$) Intake on Growth and Nutrition of Infants, Am. J. Dis. Child. **54**:726-749 (Oct.) 1937.

21. Colby, M. G.; Macy, I. G.; Poole, M. W.; Hamil, B. M., and Cooley, T. B.: Relation of Increased Vitamin B ($B_1$) Intake to Mental and Physical Growth of Infants, Am. J. Dis. Child. **54**:750-756 (Oct.) 1937.

22. Harrell, R. F.: Effect of Added Thiamine on Learning, Contribution to Education, no. 877, Teachers College, Columbia University, New York, 1943.

23. Hoobler, B. R.: Symptomatology of Vitamin B Deficiency in Infants, J. A. M. A. **91**:307-310 (Aug. 4) 1928.

24. Hoobler, B. R.: Use of Vitamin B in Diets of Infants, J. A. M. A. **96**:675-677 (Feb. 28) 1931.

showed symptoms of rigidity." This would indicate that the diets of the mothers had been inadequate in vitamin B during their pregnancies. This investigator found that "this symptom disappeared in all but 6 cases, indicating that the quantity of vitamin B needed by infants differs greatly."

Gaynor and Dennett,[25] in 1934, stated that feeding dried milk reenforced with a water extract of rice polishings influenced weight gains favorably, increased metabolic efficiency, improved nutrition and produced greater resistance to infection. Anorexia and gastrointestinal disturbances did not occur, nor did abdominal colic and constipation. Withdrawal of the rice extract resulted in immediate lowering of the growth rate.

Clements'[26] observation that breast-fed babies frequently exhibit partial thiamine deficiencies is of interest. In its first two to three months of independent life the infant uses thiamine stored in its body during intrauterine life. Ordinarily a low concentration of thiamine in a mother's milk is sufficient to provide for gain in weight but this gain may be less than the normal rate. An inadequate intake of thiamine may also produce constipation and may cause characteristic vomiting. Clements also noted that partial thiamine deficiency was not uncommon in breast-fed infants in Australia and that this was attributable to suboptimal quantities of thiamine in the milk, resulting from the mothers' low intake of thiamine.

TABLE 1.—*The Thiamine Content of Human Milk*

| Investigators | Method | Subjects | Samples | Period of Lactation | Total Thiamine, Micrograms per 100 Cc. Range | Total Thiamine, Micrograms per 100 Cc. Mean | Free Thiamine, Micrograms per 100 Cc. Range | Free Thiamine, Micrograms per 100 Cc. Mean | Comment |
|---|---|---|---|---|---|---|---|---|---|
| Donelson and Macy [15] | Rat growth | .. | Pooled | 2-11 mo. | ..... | 16.6 | | | |
| Eddy and Morris [38] | Rat growth | .. | Pooled | ..... | ..... | <20.8 | | | |
| Neuweiler, W.: Klin. Wchnschr. 17:296-298 (Feb. 26) 1938 | Thiochrome | .. | .. | 2-20 weeks | ..... | .... | Trace-13* | 10* | |
| Neuweiler [48] | ..... | .. | .. | 9-13 weeks | 4.5-19.0 | .... | 2.4-9.6 | | |
| Morgan and Haynes [18] | Rat growth | 1 | .. | 3d mo. | ..... | 11 | ..... | .... | Diet poor in thiamine |
| | | 1 | .. | 5th mo. | ..... | 32 | ..... | .... | Diet rich in thiamine |
| Widenbauer and Heckler [36] | Thiochrome | .. | .. | 1-2 days | ..... | .... | 11-24 | 16 | |
| | | | | 3-120 days | ..... | .... | 2-36 | 10.0 | |
| Utheim Toverud [16] | Thiochrome | 10 | .. | 2-4 mo. | ..... | .... | 1.7-7.85 | | |
| | (a) Jansen | .. | 11 | ..... | ..... | .... | 1.67-7.78 | 4.55 | |
| | (b) Wang and Harris | | 22 | ..... | ..... | .... | 4.1-16.7 | 10.8 | |
| Sundararajan [40] | ..... | .. | 23 | 1-4 mo. | 5.8-26.2 | 16.3 | | | |
| | | | 21 | Over 4 mo. | 6.2-31.0 | 19.5 | ..... | .... | Subjects normal |
| | | | 12 | ..... | 10.9-24.0 | 16.0 | ..... | .... | Subjects with beriberi |
| Knott [37] | Rat growth | .. | 17 | ..... | 3-18 | 9 | ..... | .... | Later called erroneously low |
| Williams, Cheldelin and Mitchell [34] | Yeast growth | 4 | 8 | 3-13 days | ..... | 9 | | | |
| Kendall [32] | Thiochrome | 63 | .. | 2-10 days | 0.0-9.0 | | | | |
| Slater and Rial [15] | Thiochrome | .. | .. | 3d week | ..... | 9.5 | | | |
| | | | | 20th week | ..... | 14.8 | | | |
| | | 40-33† | 77-57‡ | 5-37 weeks | 4.7-15.9 | 10.1 | 1.0-10.1 | 5.0 | Abnormal mothers |
| | | 29-21† | 80-61‡ | 3-38 weeks | 5.1-18.8 | 12.7 | 1.3-12.4 | 4.3 | Normal mothers |
| Knott, Kleiger and Torres-Bracamonte [32] | Microfermentation | 50 | 111 | 0-61 weeks | ..... | 15.1 | ..... | .... | Average of nursing mothers |
| | | 33 | 55 | ..... | ..... | 20.1 | ..... | .... | Mothers nursing without supplement |
| | | 8 | 18 | ..... | ..... | 9.3 | ..... | .... | Mothers nursing but also feeding a supplementary food |
| Herraiz and Herrero [35c] | ..... | 229 | 33 | 0-5 days | ..... | 19.0 | | | |

* Free plus protein-bound thiamine.
† The first figure is the number for whom the total thiamine content of the milk was determined; the second, the number for whom the free thiamine was determined.
‡ The first figure is the number of samples for total thiamine; the second, the number for free thiamine.

Table 1 demonstrates the great need for further studies on the thiamine content of mothers' milk. The few available studies in the literature have shown a variety of methods being used for the determination of thiamine. Only two investigations have included determinations of both total and free thiamine in breast milk, and observations have not been recorded for twenty-four hour collections of milk or for complete daily collections made during the initial stages of milk flow and at intervals thereafter during the lactation cycle of women whose diets are comparable in quality and known in quantity.

25. Gaynor, M. F., and Dennett, R. H.: Rice Polishings as a Source of Vitamin B Complex in Infant Feeding, J. Pediat. 4:507-513 (April) 1934.

26. Clements, M. W.: The Symptoms of Partial Vitamin $B_1$ Deficiency in Breast-Fed Infants, M. J. Australia 1:12-16 (Jan. 3) 1942.

In the present study thiamine was determined in 60 samples of milk secreted during the first ten days post partum and in 91 samples of milk secreted during the second to tenth month of lactation, obtained from mothers receiving known diets, and in 187 samples of milk produced during the first year of lactation by women on diets of their own choosing.

## METHOD

Thiamine was determined by the thiochrome method as described by Hennessy,[27] with slight modifications. As suggested by Brown, Hamm and Harrison,[28] the activated Decalso was stored under water, rather than dried. Isobutanol was recovered by treatment with Darco G-60[29] instead of redistillation from all glass.

Decalso (60 to 80 mesh) was activated by stirring a convenient amount in a beaker with 6 to 8 volumes of hot 3 per cent acetic acid. The material was treated four times, each time for fifteen to twenty minutes. Between the third and fourth washings, a treatment for twenty minutes with four volumes of hot 25 per cent postassium chloride was introduced. Finally, the Decalso was washed with boiling distilled water until a test for chloride showed none[30]; then it was stored under distilled water. A new batch was prepared every week or ten days, but the activity remained unchanged over longer periods.

Ten 15 or 20 cc. amounts of human milk (usually 15 cc.) were used for each determination. For duplicate determinations of free and total thiamine, four samples were required. Each sample was placed in a red 125 cc. Erlenmeyer flask, made to approximately 40 cc. with water and normal sulfuric acid, so that the final normality was one tenth, and autoclaved for ten minutes at 15 pounds (6.8 Kg.) of pressure. After the samples had cooled, the $p_H$ was adjusted to 4.5 with two and a half times normal sodium acetate. Duplicate samples were incubated under toluene overnight (sixteen hours) at 37 C. with approximately 20 mg. of clarase (diastase of malt, highly concentrated). The clarase was assayed for thiamine, and no correction factor was found necessary for this amount. The other 2 samples were held under toluene overnight.

The next morning all samples were made to 50 cc. and filtered through no. 1 Whatman filter paper. Forty cubic centimeters of the filtrate from each sample was passed through Decalso columns packed with hot water, with a ball of glass wool at the base of the column. The "drippings" from each column, plus the first 10 cc. of wash water, were collected in the beakers to be used for the riboflavin determination. The entire procedure was carried out in a semidarkened room. The Decalso columns were washed three times with boiling distilled water and the thiamine eluted with 15 to 20 cc. of boiling acid–potassium chloride solution (25 per cent potassium chloride made tenth-normal with respect to hydrochloric acid). The tops of the columns were not permitted to dry, to prevent oxidation of the thiamine by air.

The thiamine was oxidized to thiochrome in a small separatory funnel especially designed to be centrifuged. To 5 cc. of the potassium chloride eluate, 3 cc. of 15 per cent sodium hydroxide or 3 cc. of 0.04 per cent potassium ferrocyanide in 15 per cent sodium hydroxide was added, followed immediately by 15 cc. of isobutanol from an automatic pipet. The mixture was shaken for ninety seconds, then centrifuged to separate the two layers (about one and one-half minutes). The lower, alkaline layer was drawn off, the isobutanol dried with anhydrous sodium sulfate, which had been checked to make certain that it did not contain fluorescent substances, and the thiochrome measured in a Coleman Photofluorometer. The instrument was set before each reading with a solution of quinine sulfate containing 15 micrograms per cubic centimeter. The standard thiochrome was prepared by using 0.5 microgram of thiamine in 5 cc. of water and carrying through the oxidation step already described.

A large factor in the precision of thiamine determinations is the size of the galvanometer readings obtained from the photofluorometer. From table 2, any reading which gives a difference of 10 or more between sample and blank is also precise within 5 to 7 per cent (or ± 2.5 to 3.5 per cent).

TABLE 2.—*Reproducibility of the Results of Thiamine Analysis*

| Sample | Galvanometer Reading * | Mean, Micrograms per 100 Cc. | Standard Deviation, Micrograms per 100 Cc.† | Maximum Difference Between Duplicates, Micrograms per 100 Cc.# | Per Cent Variation ¶ |
|---|---|---|---|---|---|
| | | Free Thiamine | | | |
| Colostrum ‡ | Under 5 | 0.00572 | 0.00115 | 0.00134 | 40.0 |
| Mature milk § | Under 10 | 0.02178 | 0.00248 | 0.00334 | 22.7 |
| | 10-20 | 0.05006 | 0.00143 | 0.00203 | 5.7 |
| | | Total Thiamine | | | |
| Colostrum ‡ | Under 10 | 0.01838 | 0.00131 | 0.00187 | 14.2 |
| | 10-20 | 0.04279 | 0.00098 | 0.00145 | 4.6 |
| | Over 20 | 0.0731 | 0.0026 | 0.0047 | 7.1 |
| Mature milk § | Over 20 | 0.1451 | 0.0037 | 0.0063 | 5.1 |

* The galvanometer reading represents the value obtained when the blank reading has been subtracted.

† The standard deviation between duplicates was obtained by analysis of variance. Macy, I. G.: Nutrition and Chemical Growth in Childhood: I. Evaluation, Springfield, Ill., Charles C Thomas, Publisher, 1942, pp. 365-372.

# This is the difference one would expect if P equals 0.001 in the Chi square test.

‡ This represents twenty-four hour daily collections of milk obtained during the first ten days post partum from women on comparable and known diets.

§ This represents four to eight hour collections of milk obtained from women on self-chosen diets during mature milk flow.

¶ Two hundred times the standard deviation, divided by the mean.

## RESULTS AND COMMENT

Chart 1 shows the mean daily concentrations of total and free thiamine in the immature milk produced during the first ten days post partum. The total thiamine during the first four days of

27. Hennessy, D. J.: A Standard Thiochrome Assay for the Determination of Thiamin in Cereal Products, Cereal Chem. **2**:25-29 (May 1) 1942.

28. Brown, E. B.; Hamm, J. C., and Harrison, H. E.: Comparison of Thiamine Values by Chemical and Bioassay Methods, J. Biol. Chem. **151**:153-161 (Nov.) 1943.

29. Pader, M.: Recovery of Solvents Used in the Chemical Determination of Thiamine, Indust. & Engin. Chem. (Anal. Ed.) **15**:25 (Jan. 15) 1943.

30. Thiochrome Assay for Thiamine Hydrochloride, in Pharmacopeia of the United States of America: Twelfth Revision, Easton, Pa., Mack Printing Company, 1943, supp. 1, pp. 82-84.

lactation is low, varying from about 0.9 microgram to 2.4 micrograms per hundred cubic centimeters. From the fifth day on, the average curve continues to rise throughout the puerperium, reaching an average value of 8.1 micrograms per hundred cubic centimeters on the tenth day. Since thiamine in the blood is lower after parturition than at the end of pregnancy,[31] this may be the cause in part, at least, of the low concentration of thiamine in the early milk.

Kendall [32] studied early human milk, publishing his results as a scatter diagram in 1942. He collected 63 specimens of milk by pumping the breasts of lactating women (all ward patients) at the regular time of feeding. Aliquots of these collections were used for analysis. The thiamine values tended to increase after the first few days of lactation, and the range during the first four days post partum was similar, but the slope of the curve of concentration vs. days post partum was more gradual than in the curve shown in chart 1. On the seventh day Kendall's median value was 3.8 micrograms per hundred cubic centimeters; we found 4.6 micrograms. The difference was even larger on the ninth day, when Kendall's median value was 5.5 micrograms per hundred cubic centimeters, compared with our mean of 7.8 micrograms. Since his subjects came from a low income group, they probably were poor nutritionally, as well, whereas the subjects for the present study were multiparas with records of successful lactation and good nutrition.

Knott, Kleiger and Torres-Bracamonte [33] also analyzed human milk obtained during the first nine days of lactation. They, too, found a gradual increase in the thiamine concentration as lactation progressed. The milk during the first few days contained little thiamine, and after nine days only 1 woman had a level higher than 6 micrograms per hundred cubic centimeters. This value is somewhat lower than those obtained in this study; however, Knott's values were based on single expressions of milk rather than on twenty-four hour collections.

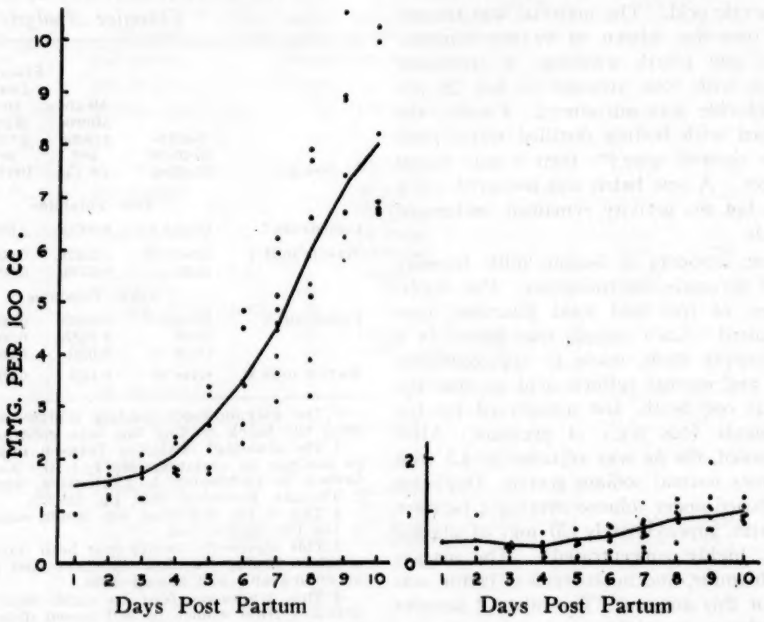



Chart 1.—Total thiamine (left) and free thiamine (right) in twenty-four hour collections of immature human milk from women receiving known diets. The curve was obtained by three point smoothing of the means.

Williams and co-workers,[34] using his yeast growth method, found an average of 9 micrograms per hundred cubic centimeters for 8 samples of milk collected during the period of three to thirteen days after parturition. His range was from less than 1 to 23 micrograms. His method of obtaining samples was not described. The

31. Gaehtgens, G., and Sauerbrey, B.: Der Einfluss der Geburt auf den Aneurin- (Vitamin $B_1$) Spiegel des Mütterlichen Blutes, Arch. f. Gynäk. **171**:425-431, 1941.

32. Kendall, N.: Thiamine Content of Various Milk, J. Pediat. **20**:65-73 (Jan.) 1942.

33. Knott, E. M.; Kleiger, S. C., and Torres-Bracamonte, F.: Factors Affecting the Thiamine Content of Breast Milk, J. Nutrition **25**:49-58 (Jan.) 1943.

34. Williams, R. J.; Cheldelin, V. H., and Mitchell, H. K.: The B Vitamin Content of Milk from Animals of Different Species, in Publication 4237, University of Texas, 1942, pp. 97-104.

results obtained by Escudero's group,[35] in Argentina, differ even more. For pooled samples collected during the first five days post partum, they obtained an average of 19 micrograms per hundred cubic centimeters. This average included values from women in a hospital admitting patients of low income families, principally, and values for a group whose nutritional background was better. The average of the first group was 8.4, that of the second 24.1, micrograms per hundred cubic centimeters. One subject, who had been taking vitamin supplements, produced milk containing 81.4 micrograms per hundred cubic centimeters, a concentration higher than any single value reported elsewhere. Their method, although not discussed in detail, was described as that of Conner and Straub.

Slater and Rial,[15] who made an extensive study of thiamine in mature milk, also present a few figures on the concentration in early milk. For four samples collected six and seven days post partum their average was 4 micrograms per hundred cubic centimeters.

Free thiamine shows only a small increase during the first ten days of lactation. The concentration is less than 1 microgram per hundred cubic centimeters for all samples collected on the first six days. The smoothed curve for the mean concentration of free thiamine shows a rise from less than 0.5 to 1.0 microgram per hundred cubic centimeters during the fourth to tenth days post partum.

Few determinations of free thiamine in early human milk have been reported in the literature. Widenbauer and Heckler,[36] in 1939, published thiamine values for human milk, but since their method omitted an incubation step, the values must represent free thiamine rather than total thiamine. They obtained a mean value of 16 micrograms per hundred cubic centimeters, with a range of 12 to 20 micrograms, for milk collected during the first two days of lactation. Since they extracted thiochrome directly from an oxidized milk sample and since colostral milk has many colored pigments in it, it is possible that they measured other compounds than thiochrome.

Slater and Rial [15] have given three values for free thiamine in milk collected on the sixth and seventh days after parturition. Their average was 3.2 micrograms per hundred cubic centimeters, a value not reached by any of the subjects in this study during the first ten days of lactation.

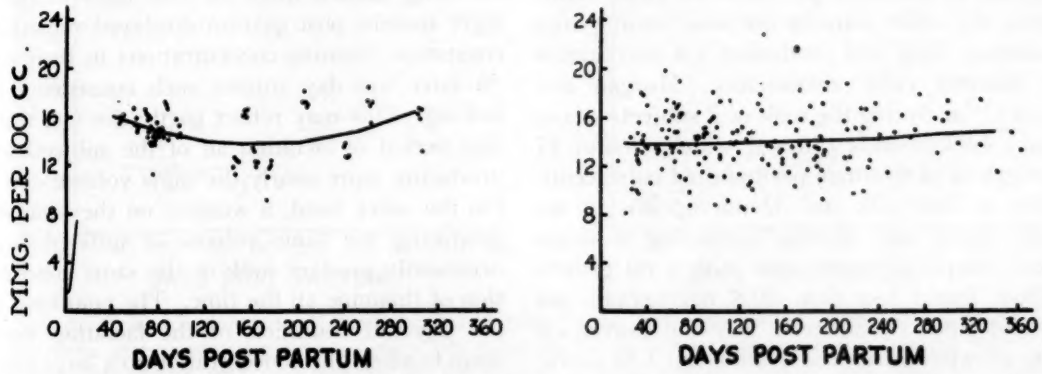



Chart 2.—Total thiamine content of mature human milk. Left: Total thiamine in twenty-four hour collections of milk from women receiving known diets. The curve for the first ten days of lactation is repeated from chart 1. The curve for mature milk was obtained by the method of least squares. Right: Total thiamine in samples from women eating self-chosen diets.

Chart 2 presents the concentrations of total thiamine as lactation progresses. Between the tenth day and the fifth week post partum the thiamine content of the milk increases to the level found in mature milk. The mean values for the twenty-four hour collections of milk from women receiving known diets (14.8 micrograms per hundred cubic centimeters) corresponds closely with the mean for the four to eight hour samples of milk from women eating diets of their own choice (14.0 micrograms per hundred cubic centimeters). The diets of the latter group, since they were self chosen, were undoubtedly inferior to those given the women whose diets were supervised. For both groups the average concentration of thiamine in the milk remained

35. (*a*) Escudero, P., and Pertusi de Esquef, L.: Estudios sobre el calostro humano: La alimentación del niño en los primeros cinco días de su vida, Rev. Asoc. argent. dietol. **2**:107-113 (April-June) 1944. (*b*) Escudero, P., and Pierangeli, E.: Influencia de la alimentación de la grávida sobre la riqueza mineral y Vitamínica del calostro, ibid. **1**:85-94 (April-June) 1943. (*c*) Herraiz, M. L., and Herrero, H. G.: Contenido de vitaminas del calostro humano, ibid. **1**:95-98 (April-June) 1943.

36. Widenbauer, F., and Heckler, F.: Ueber den Vitamin $B_1$-Gehalt der Kuh- und Frauenmilch, Ztschr. f. Kinderh. **60**:683-690, 1939.

relatively constant after the fourth week of lactation. This is in agreement with the observations of Knott [37] but not with those of Slater and Rial.[15] Knott stated that the thiamine in milk reached the level characteristic of the subject in three weeks; Slater and Rial found a gradual increase until the twentieth week.

The early work of Macy and associates [1b] indicated that human milk contained about 16.6 micrograms per hundred cubic centimeters. Slater and Rial [15] found an average of 14.8 at the twentieth week. This was their highest average weekly value. At three weeks their average value was 9.5. The over-all average for the mature milk of normal mothers was 12.7. For a group of "abnormal" mothers the average was 10.1. Knott's group found an average value of 15.1 for mature milk. This figure is apparently derived from two groups of women, one successfully nursing babies and producing 20.1 micrograms of thiamine per hundred cubic centimeters, the other nursing but also feeding supplementary food and producing 9.3 micrograms per hundred cubic centimeters. Morgan and Haynes,[18] analyzing the milk of 2 subjects whose dietary backgrounds differed widely, found 11 micrograms of thiamine per hundred cubic centimeters in one milk and 32 micrograms in the other. Eddy and Morris,[38] assaying a single pooled sample of breast milk with a rat growth method, found less than 20.8 micrograms per hundred cubic centimeters. For the conversion of rat growth figures, the relationship 3.33 micrograms = 1 international unit = 2 Chase-Sherman units, was used.[39]

Sundararajan,[40] in India, has assayed total thiamine in the milk of two groups of women, one of which displayed no evidence of beriberi, the other of which did. In the first group, the average thiamine content was 16.3 micrograms per hundred cubic centimeters for women whose children were under 4 months of age and 19.5 micrograms for women whose children were older. Surprisingly, the value for the milk of women with beriberi was not lower than these normal values. The explanation offered is that a toxic substance resulting from disturbed carbohydrate metabolism precipitates infantile beriberi, although the milk is adequate. The presence of toxic substances in human milk as a result of the disturbance of carbohydrate metabolism in beriberi has been discussed by Fehily.[41] Complete proof is still lacking, but pyruvic acid, methylglyoxal and other intermediary products of carbohydrate metabolism have been found in human milk.[42] However, Fehily assumed that the milk was also low in thiamine.

It is true that women on similar diets do not produce exactly the same concentrations in their milk all of the time, perhaps owing to individual variation in absorption or in requirements. There are some investigators who believe thiamine synthesized by intestinal flora supplements the dietary intake.[43] Others have refuted this claim and offer evidence that thiamine is not absorbed from the large intestine.[44] However, 10 women receiving known diets for five days at two to three months post partum displayed remarkably consistent thiamine concentrations in their milk. At later five day studies such consistence was lacking. This may reflect partly the fact that at that period of lactation all of the subjects were producing more nearly the same volume of milk. On the other hand, a woman, on the same diet producing the same volume of milk, does not necessarily produce milk of the same concentration of thiamine all the time. The emotional and the physical condition of the lactating woman seem to affect the concentration to a large extent. There is also evidence that the concentration drops quite rapidly when the supply of milk is diminishing. In the present study neither the volume of milk per day (200 to 1100 cc.) nor the volume per expression (50 to 450 cc.) was related to the concentration. However, the diet showed a clearcut relationship to the thiamine in human milk. If the diet included pork, one of the richest sources of thiamine, the concentration of thiamine in the milk increased. If the diet

37. Knott, E. M.: Thiamin Content of Milk in Relation to Vitamin $B_1$ Requirement of Infants, Am. J. Pub. Health **32**:1013-1017 (Sept.) 1942.

38. Eddy, W. H., and Morris, S. G.: A Study of the Effect of Preserving Methods on Human Milk, J. Pediat. **4**:208-215 (Feb.) 1934.

39. Williams, R. R., and Spies, T. D.: Vitamin $B_1$ (Thiamin) and Its Use in Medicine, New York, The Macmillan Company, 1938, p. 221.

40. Sundararajan, A. R.: The Vitamin $B_1$ Content of Human Milk, Indian J. M. Research **29**:567-573 (July) 1941.

41. Fehily, L.: Infantile Beriberi in Hong Kong, Caduceus **19**:78-93, 1940; Human-Milk Intoxication Due to $B_1$ Avitaminosis, Brit. M. J. **2**:590-592 (Nov. 4) 1944.

42. Platt, B. S., and Lu, G. D.: CLXXXVI. Studies on the Metabolism of Pyruvic Acid in Normal and Vitamin $B_1$ Deficient States: IV. The Accumulation of Pyruvic Acid and Other Carbonyl Compounds in Beri-Beri and the Effect of Vitamin $B_1$, Biochem. J. **33**:1525-1537 (Oct.) 1939.

43. Najjar, V. A., and Holt, L. E.: The Biosynthesis of Thiamine in Man, J. A. M. A. **123**:683-684 (Nov. 13) 1943.

44. Alexander, B., and Landwehr, G.: The Role of Fecal Thiamine and Cocarboxylase on Human Nutrition, Science **101**:229-230 (March 2) 1945.

varied little in thiamine from day to day, the thiamine in the milk remained constant.

Free thiamine in mature milk, in contrast to total thiamine, shows slowly rising values as lactation progresses (chart 3). Since total thiamine is relatively constant, the percentage of free thiamine increases. The average values for free thiamine are 6.7 (standard deviation, 3.5) micrograms per hundred cubic centimeters for milk from the women on known diets and 4.7 (standard deviation, 2.5) micrograms for the survey samples of milk. The average percentage of free thiamine is 37. This is interesting in comparison with the 34 per cent which Slater and Rial [15] obtained as their average for mature milk. They concluded:

> The percentage of free thiamine varied considerably from sample to sample; but there seemed to be a general tendency for the amount of free thiamine in human milk to remain fairly constant during the first three months. . . . Towards the end of lactation, the percentage of free thiamine greatly increased.

The percentage values in this study ranged from nearly 8.8 to 100, with most of the values falling between 10 and 90 per cent. Slater and Rial found a range of 13 to 100 per cent, with the usual values falling between 15 and 50 per cent. Neuweiler [45] stated that 50 to 80 per cent of the total thiamine occurs in the free state. In this study, for the women on known diets the percentage of free thiamine increased from 11 on the tenth day to 36 at three months, 46 at six months, 71 at eight months and 94 at ten months. These values are slightly higher than those for the women on self-chosen diets because the eighth and tenth month values were weighted by the value obtained for 1 subject whose percentage of free thiamine was unusually high.

The mean values and the ranges of the means for total and free thiamine in the milk of both groups of women in the present study are given in table 3. Utheim Toverud [16] measured free thiamine in milk produced two to four months after parturition. Using the Jansen method, she obtained an average of 4.55, with a range of 1.67 to 7.78 micrograms per hundred cubic centimeters. At the same time, she also used the Wang and Harris modification of the thiochrome method and obtained an average of 10.8, with a range of 4.1 to 16.7 micrograms per hundred cubic centimeters. The results of this study, made during the same period of lactation, agree well with her first results.

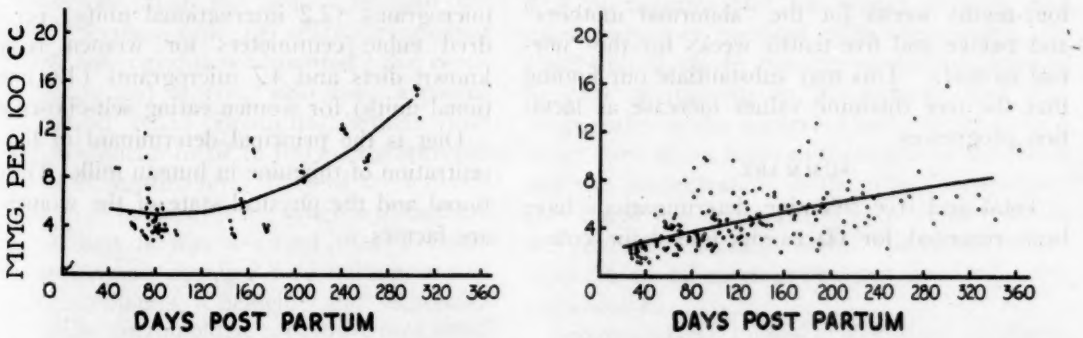



Chart 3.—Free thiamine content of mature human milk. Left: Free thiamine in twenty-four hour collections of milk from women receiving known diets. The curve for the first ten days of lactation is repeated from chart 1. The curve for mature milk was obtained by the method of least squares. Right: Free thiamine in samples from women eating self-chosen diets.

Widenbauer and Heckler [36] found an average of 10 micrograms per hundred cubic centimeters and a range of 2 to 36, for milk collected three

TABLE 3.—*Total and Free Thiamine Contents of Human Milk*

| Days Post Partum | Total Thiamine: Subjects | Total Thiamine: Samples* | Total Thiamine: Mean, Micrograms per 100 Cc. | Total Thiamine: Range, Micrograms per 100 Cc. | Free Thiamine: Subjects | Free Thiamine: Samples* | Free Thiamine: Mean, Micrograms per 100 Cc. | Free Thiamine: Range, Micrograms per 100 Cc. |
|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 2 | 1.48 | 0.92-2.05 | 1 | 1 | 0.42 | |
| 2 | 5 | 5 | 1.51 | 1.24-1.84 | 3 | 3 | 0.53 | 0.27-0.90 |
| 3 | 6 | 6 | 1.62 | 1.23-2.08 | 5 | 5 | 0.29 | 0.22-0.33 |
| 4 | 6 | 6 | 1.96 | 1.70-2.40 | 5 | 5 | 0.30 | 0.10-0.56 |
| 5 | 6 | 6 | 2.50 | 1.72-3.39 | 5 | 5 | 0.36 | 0.12-0.62 |
| 6 | 6 | 6 | 3.54 | 2.32-4.86 | 6 | 6 | 0.50 | 0.20-0.70 |
| 7 | 9 | 9 | 4.65 | 3.10-6.21 | 9 | 9 | 0.60 | 0.19-1.16 |
| 8 | 7 | 7 | 5.68 | 3.20-7.87 | 7 | 7 | 0.86 | 0.38-1.20 |
| 9 | 7 | 7 | 7.77 | 5.80-10.52 | 7 | 7 | 1.04 | 0.61-1.90 |
| 10 | 6 | 6 | 8.12 | 6.68-10.22 | 6 | 6 | 0.93 | 0.74-1.23 |
| 45-306 | 10 | 90 | 14.8 | 9.1 -18.4 | 10 | 82 | 6.67 | 2.8 -15.6 |
| 15-362 | 65 | 187† | 14.0 | 8.1 -22.7 | 67 | 177† | 4.66 | 1.2 -15.8 |

* All samples except those noted represent complete twenty-four hour collections of milk.

† These samples were complete expressions of milk secreted in four to eight hours.

45. Neuweiler, W.: Bemerkungen über den Vitamin $B_1$-Gehalt der Milch, Klin. Wchnschr. **20**:1072 (Oct. 25) 1941.

to one hundred and twenty days post partum. That value is higher than the present results indicate. Neuweiler [45] in his second study of thiamine in human milk measured both free and total thiamine and obtained values for free thiamine ranging from 2.4 to 9.6 micrograms per hundred cubic centimeters.

Slater and Rial found an average of 4.3 micrograms per hundred cubic centimeters, with a range of 1.3 to 12.4, for milk collected from "normal mothers" in the interval three to thirty-eight weeks post partum. The average value of the free thiamine in the milk from "abnormal mothers" was 5.0, with a range of 1.0 to 10.1. This somewhat higher average of free thiamine for women whose total thiamine values were low (10.1 compared with 12.7) may be due to the fact that the average number of weeks of lactation was greater in this group—twenty-one and four-tenths weeks for the "abnormal mothers" and twelve and five-tenths weeks for the "normal mothers." This may substantiate our finding that the free thiamine values increase as lactation progresses.

## SUMMARY

Total and free thiamine determinations have been recorded for 60 twenty-four hour collections of milk obtained during the first ten day post partum and 90 collections obtained two t ten months post partum from mothers receivin diets of equal quality and known quantity. Com parable values are given for 187 samples repre senting complete expressions of the milk secrete in four to eight hours by 65 women during th first year of lactation, while they were consumin diets of their own choice.

During the first days of lactation human mil contains little thiamine. The concentration o total thiamine increases throughout the first fe weeks of lactation to a value in mature huma milk of 14.0 to 14.7 micrograms (4.7 to 4. international units) per hundred cubic centi meters.

Free thiamine in mature milk does not hav a constant value but increases gradually durin lactation. The mean values found were 6. micrograms (2.2 international units) per hun dred cubic centimeters for women receivin known diets and 4.7 micrograms (1.6 interna tional units) for women eating self-chosen diet

Diet is the principal determinant of the con centration of thiamine in human milk. The emo tional and the physical state of the woman als are factors.

# HUMAN MILK STUDIES

## XXIV. FREE AND TOTAL RIBOFLAVIN CONTENTS OF COLOSTRUM AND MATURE HUMAN MILK

CHARLOTTE E. RODERUCK, M.S.; MARGARET N. CORYELL, PH.D.; HAROLD H. WILLIAMS, PH.D., AND ICIE G. MACY, PH.D.

DETROIT

Early investigators found that cow's milk ntains a considerably larger amount of ribo- avin, or the pellagra-preventative factor as it as then called, than does human milk, but it as not until 1934 that quantitative measure- ents were made. At that time Macy and asso- ates,[1] using a pooled sample of mature mother's ilk, estimated that the milk contained 0.2 ourquin-Sherman unit per cubic centimeter 40 to 60 micrograms per hundred cubic centi- eters. Eddy and Morris,[2] also using a pooled mple, found 0.333 Bourquin-Sherman unit r cubic centimeter or 67 to 100 micrograms per ndred cubic centimeters. Both of these deter- inations were made with the rat growth method. or conversion it was assumed that 1 Bour- in-Sherman unit equals 2 to 3 micrograms of boflavin. Williams, Cheldelin and Mitchell,[3] nploying the microbiologic procedure on a small mber of samples of milk collected three to irteen days post partum, found an average of 3 micrograms per hundred cubic centimeters. Argentina, Escudero's group[4] assayed 33 pooled samples of milk collected during the first five days post partum and found 24.16 micrograms per hundred cubic centimeters.

Randoin and Raffy[5] compared the riboflavin in human milk in France in 1940 with that in 1942, to ascertain the effect of the food rationing program there. In 1940 the summer average was 32.4 micrograms and the winter average 27.5 micrograms per hundred cubic centimeters. By 1942 the riboflavin concentration had dropped to 23.0 micrograms in the summer and 20.1 micrograms in the winter. These results show a direct correlation between the intake of riboflavin and the concentration in the milk. Neuweiler[6] also found a correlation between dietary intake and concentration in the milk. His values ranged from 16 to 52 micrograms per hundred cubic centimeters.

Sundararajan,[7] in India, measured riboflavin on the samples used for assay of thiamine. He obtained an average of 58.0 micrograms per hundred cubic centimeters for women who were free from beriberi and whose period of lactation did not exceed four months. The range was 19.6 to 80.0 micrograms. For women who had been lactating more than four months he found an average of 47.6 micrograms with a range of 12.6 to 100.0. The riboflavin content of milk from women with beriberi was unaffected by the deficiency of thiamine, averaging 48.0 micrograms, with a range of 33.0 to 66.0 micrograms, per hundred cubic centimeters.

From the Research Laboratory, Children's Fund of ichigan.

1. (*a*) Donelson, E. G., and Macy, I. G.: Human ilk Studies: XII. The Vitamin B and Vitamin G ontent Before and During Maternal Consumption of east, J. Nutrition **7**:231-249 (Feb.) 1934. (*b*) McCosh, S.; Macy, I. G., and Hunscher, H. A.: Human Milk tudies: VI. Vitamin Potency as Influenced by Supple- enting the Maternal Diet with Yeast, J. Biol. Chem. :1-13 (Jan.) 1931.

2. Eddy, W. H., and Morris, S. G.: A Study of e Effect of Preserving Methods on Human Milk, J. ediat. **4**:208-215 (Feb.) 1934.

3. Williams, R. J.; Cheldelin, V. H., and Mitchell, K.: The B Vitamin Content of Milk from Animals Different Species, in Studies on the Vitamin Con- nt of Tissues, Publication 4237, University of Texas, 42, p. 97.

4. (*a*) Escudero, P., and Pertusi de Esquef, L.: studios sobre el calostro humano; la alimentación del ño en los primeros cinco diás de su vida, Rev. Asoc. gent. dietol. **2**:107-113 (April-June) 1944. (*b*) Escu- ro, P., and Pierangeli, E.: Influencia de la alimenta- ón de la grávida sobre la riqueza mineral y vitamínica l calostro, ibid. **1**:85-94 (April-June) 1943. (*c*) Herraiz, M. L., and de Alvarez Herrero, H. G.: Contenido de vitaminas del calostro humano, ibid. **1**:95-98 (April-June) 1943.

5. Randoin, L., and Raffy, A.: Significant Decrease in the Vitamin $B_2$ Content of Breast Milk During the Present Food Rationing, Bull. Acad. de méd., Paris **127**:12-14, 1943; abstracted, Chem. Abstr. **38**:5538, 1944.

6. Neuweiler, W.: Ueber den Flavingehalt der Frauenmilch, Klin. Wchnschr. **16**:1348-1350 (Sept. 25) 1937.

7. Sundararajan, A. R.: The Vitamin $B_1$ Content of Human Milk, Indian J. M. Research **29**:567-573 (July) 1941.

The values recorded in the literature are recorded in table 1.

Since riboflavin is part of the enzyme systems concerned in the metabolism of carbohydrates [8] and amino acids,[9] it is of importance to find what amounts are present in human milk and what changes occur during lactation. The maternal requirement, as well as the infant's, can be studied from such data. Sure [10] has shown that rats increase their intake of food two to three times during lactation but that their riboflavin requirement is increased five to six times. The normal human requirement, and some have questioned whether there is a requirement,[11] is still under discussion, but there seems to be evidence that the need for riboflavin is greater during tissue regeneration and increased metabolic activity.[12] Riboflavin has also been shown to have a "sparing action" toward thiamine [13] and seems to be related to the metabolism of nicotinic acid.[14]

As a part of the studies described in a preceding paper,[15] riboflavin was determined in twenty-four hour collections of milk from women receiving known diets, planned by a dietitian, and in four to eight hour collections from women eating self-chosen diets.

## METHOD

By following a modified Conner and Straub [16] procedure, it was possible to measure riboflavin in the sample used for the determination of thiamine. Since there were samples extracted with tenth-normal sulfuric acid, as well as samples extracted with acid and then incubated with clarase (diastase of malt, highly concentrated), it was possible to separate the riboflavin into two fractions, a free riboflavin fraction and a free plus combined riboflavin fraction.

After thiamine had been removed on the activated Decalso columns, the milk filtrate plus the first 10 cc. of wash water was passed through a Florisil column which had been packed with hot water, a ball of glass wool being used at the base of the column. Florosil needs no special treatment to activate it and possesses the useful property that only free riboflavin is adsorbed on it. This made it possible to determine both free and total riboflavin, for combined riboflavin has the same fluorescent properties as free riboflavin under ultraviolet rays.[17] Following the adsorption of the free riboflavin

TABLE 1.—*Values for Riboflavin in Human Milk as Recorded in the Literature*

| Investigator | Method | Subjects | Samples | Period of Lactation | Total Riboflavin, Micrograms per 100 Cc. Mean | Range |
|---|---|---|---|---|---|---|
| Donelson and Macy [1a] | Rat growth | .. | Pooled | ..... | 50 * | ..... |
| Eddy and Morris [2] | Rat growth | .. | Pooled | ..... | 83 * | ..... |
| Neuweiler [6] | Chemical | .. | ..... | ..... | .... | 16 - 32 |
| Williams, Cheldelin and Mitchell [3] | Microbiologic | 4 | 8 | 3 to 13 days | 38 | ..... |
| Sundararajan [7] | Fluorometric | .. | 20 | Under 4 mo. | 58.0 | 19.6- 80.0 |
| | | | 23 | Over 4 mo. | 47.6 | 12.6-100.0 |
| | | | 9 | 7 to 150 days | 48.0 | 33.0- 66.0 |
| Escudero and co-workers [4] | Chemical | .. | Pooled | 1 to 5 days | 24.16 | |
| Randoin and Raffy [5] | ..... | .. | ..... | 1940 | 32.4 summer<br>27.5 winter | |
| | | | | 1942 | 23.0 summer<br>20.1 winter | |

* The number of Bourquin-Sherman units was converted to number of micrograms with the factor 2.5.

8. Laszt, L., and Verzár, F.: Die Störungen des Kohlenhydrat-Stoffwechsel bei Ausfall der Nebennierenrinde und ihr Zusammenhang mit dem Na-Stoffwechsel, Biochem. Ztschr. **292**:159-173, 1937. Verzár, F.; Hüber, H., and Laszt, L.: Die Bindung des Lactoflavins als Lactoflavinphosphosäure in Körper nach Nebennierenextirpation, ibid. **292**:152-158, 1937.

9. Warburg, O., and Christian, W.: Isolierung der prosthetischen Gruppe der *d*-aminosäureoxydase, Biochem. Ztschr. **298**:150-168, 1938. Krebs, H. A.: Untersuchungen über den Stoffwechsell der Aminosäuren im Tierkörper, Ztschr. f. physiol. Chem. **217**:191-227, 1933. Straub, F. B.: Coenzyme of the *d*-Amino-Acid Oxidase, Nature, London **141**:603-604 (April 2) 1938.

10. Sure, B.: Quantitative Requirements of the Components of the Vitamin B Complex for Lactation and Growth of Nursing Young of the Albino Rat, J. Nutrition **19**:57-69 (Jan.) 1940.

11. Keys, A.; Henschel, A. F.; Mickelsen, O.; Brozek, J. M., and Crawford, J. H.: Physiological and Biochemical Functions in Normal Young Men on a Diet Restricted in Riboflavin, J. Nutrition **27**:165-178 (Feb.) 1944. Najjar, V. A.; Johns, G. A.; Medairy, G. C.; Fleischmann, G., and Holt, L. E.: The Biosynthesis of Riboflavin in Man, J. A. M. A. **126**:357-358 (Oct. 7) 1944.

12. Copping, A. M.: Some Aspects of Riboflavin Nutrition in Man, Nutrition Abstr. & Rev. **14**:433-44 (Jan.) 1945. Sure.[10]

13. Ellis, L. N., and Zmachinsky, A.: The Sparing Action of Lactoflavin on Vitamin $B_1$, Science **86**:245-246 (Sept. 10) 1937.

14. Vilter, R. W.; Vilter, S. P., and Spies, T. D. Determination of the Codehydrogenases I and II (Cozymase) in the Blood of Diabetics in Severe Acidosis Am. J. M. Sc. **197**:322-326 (March) 1939.

15. Macy, I. G.; Williams, H. H.; Pratt, J. P., and Hamil, B. M.: Human Milk Studies: XIX. Implications of Breast Feeding and Their Investigation, Am. J. Dis. Child., this issue, p. 135.

16. Conner, R. T., and Straub, G. J.: Combined Determination of Riboflavin and Thiamine in Food Products, Indust. & Engin. Chem. (Anal. Ed.) **13**: 380-384 (June 15) 1941.

17. Rosner, L.; Lerner, E., and Cannon, H. J.: The Determination of Free and Combined Riboflavin, in Proceedings of the American Chemical Society, September 1944, p. 5A.

he Florosil columns were washed three times with hot ater. Twenty cubic centimeters of hot acetic acid–yridine solution (20 per cent pyridine in 2 per cent cetic acid) was used to elute the riboflavin. The eluate, rotected from direct light, was treated with a few rops of 4 per cent potassium permanganate (enough maintain a pink color) to oxidize any impurities ill present. The excess permanganate was oxidized ith hydrogen peroxide (about 6 per cent). After the uates were adjusted to a convenient volume with ater, the fluorescence was read with appropriate filters a Coleman photofluorometer, Model 12. Blanks were btained by destroying the riboflavin with 0.2 to 0.3 cc. a solution of sodium hydrosulfite and sodium bicar-onate and rereading. Standards for comparison were ade up in an acetic acid–pyridine solution and carried rough the permanganate–hydrogen peroxide step with e samples. These standards when treated with the

TABLE 2.—*Reproducibility of the Results of Riboflavin Analyses*

| Sample | Galvanometer Reading * | Mean, Micrograms per 100 Cc. | Standard Deviation, Micrograms per 100 Cc.† | Maximum Difference Between Duplicates, Micrograms per 100 Cc.# | Per Cent Variation ¶ |
|---|---|---|---|---|---|
| **Free Riboflavin** | | | | | |
| Colostrum ‡ | 10-20 | .1964 | .0163 | .0228 | 16.6 |
| Mature milk § | 10-20 | .2026 | .0111 | .0162 | 10.9 |
| Colostrum ‡ | Over 20 | .2531 | .0152 | .0214 | 12.0 |
| Mature milk § | Over 20 | .3770 | .0114 | .0166 | 6.1 |
| **Total Riboflavin** | | | | | |
| Colostrum ‡ | Over 20 | .3458 | .0086 | .0130 | 5.0 |
| Mature milk § | Over 20 | .4329 | .0087 | .0131 | 4.0 |

* The galvanometer reading represents the value obtained en the blank reading has been subtracted.
† The standard deviation between duplicates was obtained analysis of variance.
# This is the difference one would expect if P equals 0.001 in e chi square test.
‡ This represents twenty-four hour daily collections of milk tained during the first ten days post partum from women on own diets.
§ This represents four to eight hour samples of milk obtained ring mature milk flow from women on self-chosen diets.
¶ Two hundred times standard deviation divided by the an.

dium hydrosulfite–sodium bicarbonate solution gave a ank reading of zero.

The precision of the riboflavin determinations has been aluated statistically. Representative samples were vided into groups based on the magnitude of the galnometer reading after the blank reading had been btracted. From table 2 it is apparent that free riboavin values for colostrum are not reproduced as readily those for mature milk, owing to the fact that colosum samples give higher and more inconsistent blank adings. A yellowish brown color is characteristic of e colostral milk filtrates obtained after removal of the otein, and none of the purification steps in the produre eliminate the colored substance. It disappears, t only temporarily, as a result of the permanganate–drogen peroxide treatment, and again, as a result of e addition of the hydrosulfite–bicarbonate solution. ee riboflavin, since it gives a smaller value than tal riboflavin, is affected more by this interference. otal riboflavin, however, can be duplicated within per cent.

We have used the microbiologic method also, obtain- results approximately 20 per cent higher than those obtained with the chemical method, but they seem less reliable, since determinations on the same sample could not be duplicated from day to day. This difficulty has been encountered by others.[18] For 1 sample of milk the values obtained microbiologically ranged from 49 to 64 micrograms per hundred cubic centimeters in twenty-six determinations over a period of four weeks, a variation of 27 per cent. The values obtained with the chemical method in a similar experiment varied only 5 per cent. For these reasons, only the chemical determinations are presented.

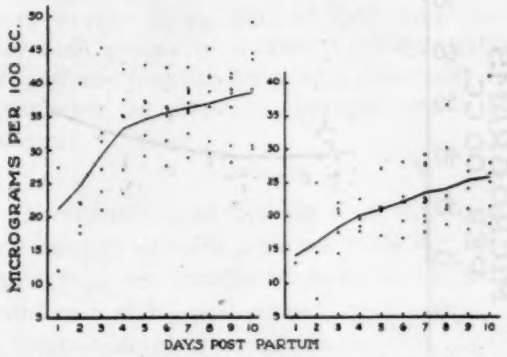



Chart 1.—Total riboflavin (left) and free riboflavin (right) in twenty-four hour collections of immature human milk obtained from women receiving the known diet. The curve was obtained by three point smoothing of the means.

TABLE 3.—*Total and Free Riboflavin Contents of Human Milk*

| Days Post Partum | Total Riboflavin: Subjects | Total Riboflavin: Samples* | Total Riboflavin: Mean, Micrograms per 100 Cc. | Total Riboflavin: Range, Micrograms per 100 Cc. | Free Riboflavin: Subjects | Free Riboflavin: Samples* | Free Riboflavin: Mean, Micrograms per 100 Cc. | Free Riboflavin: Range, Micrograms per 100 Cc. |
|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 2 | 19.6 | 12.0-27.3 | 1 | 1 | 13.1 | ..... |
| 2 | 5 | 5 | 24.0 | 17.5-40.0 | 3 | 3 | 15.8 | 7.7-25.0 |
| 3 | 6 | 6 | 30.2 | 21.0-45.3 | 5 | 5 | 19.1 | 14.4-22.0 |
| 4 | 6 | 6 | 33.4 | 23.6-42.0 | 5 | 5 | 19.6 | 17.7-22.5 |
| 5 | 6 | 6 | 35.6 | 29.1-42.6 | 5 | 5 | 21.4 | 17.0-27.0 |
| 6 | 6 | 6 | 34.8 | 27.5-42.3 | 6 | 6 | 21.8 | 16.6-28.0 |
| 7 | 9 | 9 | 36.5 | 28.8-42.4 | 9 | 9 | 23.1 | 17.6-29.1 |
| 8 | 7 | 7 | 37.5 | 29.4-45.0 | 6 | 6 | 25.2 | 18.7-36.6 |
| 9 | 7 | 7 | 36.4 | 28.0-41.3 | 6 | 6 | 23.9 | 18.0-28.2 |
| 10 | 6 | 6 | 39.4 | 30.0-49.0 | 6 | 6 | 26.7 | 18.8-40.8 |
| 45-306 | 9 | 86 | 41.3 | 28.5-62.2 | 9 | 80 | 28.3 | 15.7-50.0 |
| 15-362 | 67 | 187† | 35.4 | 19.8-79.0 | 65 | 177† | 22.3 | 10.5-61.8 |

* All samples except those marked by a dagger represent complete twenty-four hour collections of milk.
† These samples were complete expressions of the milk secreted in four to eight hours.

## RESULTS AND COMMENT

The values for total and free riboflavin in twenty-four hour collections of milk obtained from the women on known diets during the first ten days post partum are plotted in chart 1. The smoothed curve shows a rapid rise in the riboflavin content of human milk during the first

18. Andrews, J. S.: Report of 1942-3 Methods of Analysis Subcommittee on Riboflavin Assay, Cereal Chem. **20**:613-625, 1943.

half of the puerperium and a slight increase from the fifth to the tenth day post partum. This is in contrast to the curve for thiamine,[19] which showed the greatest increase during the second five days post partum. Free riboflavin constitutes a slightly increasing percentage of the total, showing a more gradual increase during the first ten days of lactation than does total riboflavin.

trations have attained approximately the level found in mature human milk. The effect of liver on the concentration of riboflavin in immature milk is noteworthy: On the fifth and tenth days the meal at noon included a serving of liver.[20] The mean daily riboflavin contents of the milk show a possible influence of this food on the values for the fifth day and a greater effect on those for the tenth day.

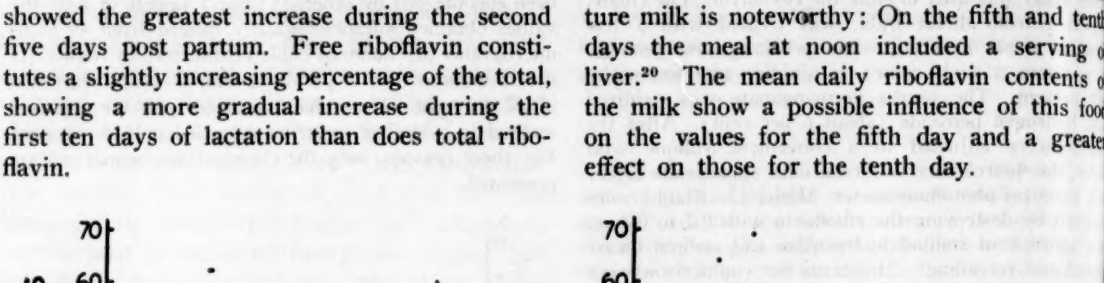



Chart 2.—Total riboflavin in mature human milk. Left: Total riboflavin in twenty-four hour collections of milk obtained from women receiving known diets. The curve for the first ten days of lactation is repeated from chart 1. The curve for mature milk was obtained by the method of least squares. Right: Total riboflavin in samples from women eating self-chosen diets.

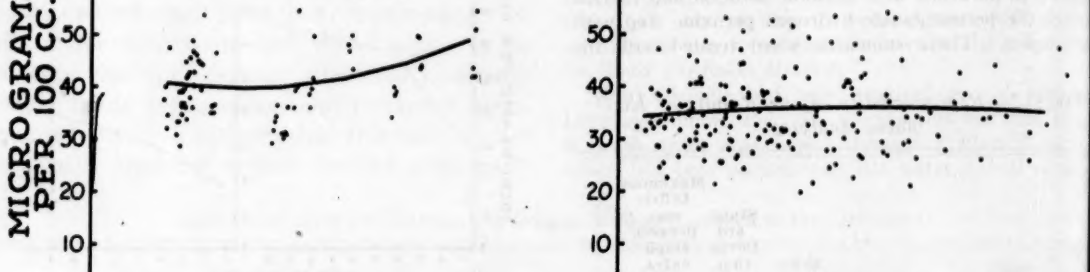



Chart 3.—Free riboflavin in mature human milk. Left: Free riboflavin in twenty-four hour collections of milk obtained from women receiving known diets. The curve for the first ten days of lactation is repeated from chart 1. The curve for mature milk was obtained by the method of least squares. Right: Free riboflavin in samples from women eating self-chosen diets.

The mean daily values given in table 3 show that both total and free riboflavin almost exactly double in concentration during the first ten days of lactation. By the tenth day the concen-

There are few figures available for comparison with ours. In Argentina, Escudero and Pertus

19. Roderuck, C. E.; Williams, H. H., and Macy, I. G.: Human Milk Studies: XXIII. Free and Total Thiamine Contents of Colostrum and Mature Human Milk, Am. J. Dis. Child., this issue, p. 162.

20. Kaucher, M.; Moyer, E. Z.; Richards, A. J. Williams, H. H.; Wertz, A. L., and Macy, I. G. Human Milk Studies: XX. The Diet of Lactating Women and the Collection and Preparation of Food and Human Milk for Analysis, Am. J. Dis. Child., this issue, p. 142.

le Esquef [4a] obtained an average of 24.16 micrograms of total riboflavin per hundred cubic centimeters for pooled milk samples collected during he first five days of lactation. In our study, the verage value rose from 19.6 micrograms on the irst day to 24.0 on the second, 30.2 on the hird, 33.4 on the fourth and 35.6 on the fifth ay. These figures are in the range of Escuero's figures, which is interesting, since he did ot use twenty-four hour collections.

The average value for total riboflavin in 86 wenty-four hour expressions of mature milk ollected from 9 women eating known diets chart 2) is 41.3 micrograms per hundred cubic entimeters, with a standard deviation of 7.9. he data from 67 women eating diets of their wn choice, from whom 187 four to eight hour ollections were obtained (chart 2), show an verage value of 35.4 micrograms with a standard eviation of 8.4. The difference between the roup averages was shown statistically to be ignificant by the Fisher "t" test. Variations ttributable to differences in volume or season ould not be found in the data, but there was a efinite relation between the amount of riboavin in the diet and the amount secreted in the ilk. On the fifth day of each five day study, ver was included in the dietary. Invariably, he concentration of riboflavin in that day's milk ncreased noticeably. When liver was one of the oods chosen by any of the mothers whose diet as not analyzed, the milk always exhibited a igher concentration of riboflavin. These inreases were greater than those noted for immaure milk.

Free riboflavin (chart 3) is approximately a constant percentage of the total (chart 2) throughout the period of lactation studied. This percentage varies from 43 to 86, averaging approximately two thirds of the total. In 1937, Neuweiler had found that the flavin of human milk was almost completely dialyzable, but he gave no exact figures. The concentration of riboflavin in the twenty-four hour collections of milk from the women receiving known diets was significantly greater than that in the samples from women eating diets of their own choice. For both groups of women the concentrations of total and free riboflavin were essentially constant from the second to the tenth month post partum.

## SUMMARY

Determinations of free and total riboflavin in 333 samples of milk collected from 80 women throughout ten months of lactation, beginning with the first day post partum, revealed that the concentration of total riboflavin rises rapidly during the first five days of lactation and slowly during the next five days. The concentrations of both free and total riboflavin attain the levels characterizing mature milk by the tenth day. The average value of total riboflavin in mature human milk was 41.3 micrograms per hundred cubic centimeters for women on known diets, planned by a dietitian, and 35.4 micrograms for women on self-chosen diets. Free riboflavin composed from 43 to 86 per cent of the total riboflavin in mature human milk.

# HUMAN MILK STUDIES

## XXV. ASCORBIC ACID AND DEHYDROASCORBIC ACID IN COLOSTRUM AND MATURE HUMAN MILK

BERTHA MUNKS, B.S.; ABNER ROBINSON, M.S.;
HAROLD H. WILLIAMS, PH.D., AND ICIE G. MACY, PH.D.
WITH THE ASSISTANCE OF MARJORIE LESHER, M.S.; MARY ELLEN HARMON, B.S.; JAMES K. BRODY, M.S.; JANE ANDERSON, PH.D., AND ROSEMARY RUST, B.S.
DETROIT

Approximately thirty studies of the ascorbic acid concentration in human milk have been made in various parts of the world, including the United States, England, India, China, Japan and the Philippines. The vitamin C concentration in human milk for peoples of these countries, living in different environments and consuming many different types of diets, ranges from less than 1 to 9 mg. per hundred cubic centimeters (table).

Only two studies have been made in the United States: Selleg and King [1] reported the vitamin C concentration in human milk, obtained from the mothers three to ten days post partum. Ingalls, Draper and Teel [2] reported analyses made of the milk of mothers four to five, seven to eight and fourteen days post partum, also a total of nine vitamin C determinations made after the women had been lactating for one to eight months. Selleg and King obtained their milk samples one to three times a day. Ingalls and co-workers did not mention the time of collection of the milk in the hospital and their 9 samples of mature milk represent milk expressed from the breasts after the mothers had nursed their babies.

These pioneer studies of the nourishment of young infants demonstrated the need for more intensive studies of the composition of human milk obtained from healthy, normal women living under conditions approximating those under which infants are successfully breast fed. For such an investigation the total intake of nutriments by the mother should be determined, together with the amounts of nutriments contained in the milk secreted at different periods in a single day and the nutriments supplied for the infant in the complete twenty-four hour production of milk, at various stages in lactation.[2a]

### PROCEDURE

In the present study, reduced or total ascorbic acid or both have been determined in 60 twenty-four hour collections of milk secreted during the first ten days post partum and in 90 twenty-four hour collections obtained during periods of five consecutive days at intervals in lactation. During these five day periods the women were ingesting diets of known composition, planned by a dietitian.[3] The variations in the concentration of reduced ascorbic acid have been determined in samples of milk representing complete expressions from 3 of the women at four hour intervals throughout the day. Also, analyses for reduced and total ascorbic acid, or total ascorbic acid only, were made on 179 samples of milk from 66 women, each sample representing the secretion during four to eight hours when the women were eating diets of their own choice. The amount of ascorbic acid oxidized to dehydroascorbic acid was studied.

The samples from the twenty-four hour milk collections included aliquots from each four hour collection. An equal volume of 4 or 6 per cent metaphosphoric acid solution (depending on the method used) was added to each aliquot immediately after it was measured from the total amount of milk expressed. It was necessary to add the metaphosphoric acid in solution because the milk volume could not be anticipated and small weighings of solid metaphosphoric acid would be impossible in the hospital and the homes. The samples from the women eating self-chosen diets consisted of 10 cc. of milk taken from the amounts obtained by complete expression of the breasts and were preserved with 1.2 Gm. of solid metaphosphoric acid. In the laboratory the milk samples were diluted and allowed to stand in the ice box before

From the Research Laboratory, Children's Fund of Michigan.

1. Selleg, I., and King, C. G.: The Vitamin C Content of Human Milk and Its Variation with Diet, J. Nutrition **11**:599-605 (June) 1936.

2. Ingalls, T. H.; Draper, R., and Teel, H. M.: Vitamin C in Human Pregnancy and Lactation: II. Studies During Lactation, Am. J. Dis. Child. **56**:1011-1019 (Nov.) 1938.

2a. Macy, I. G.; Williams, H. H.; Pratt, J. P., and Hamil, B. M.: Human Milk Studies: XIX. Implications of Breast Feeding and Their Investigation, Am. J. Dis. Child., this issue, p. 136.

3. Kaucher, M.; Moyer, E. Z.; Richards, A. I.; Williams, H. H.; Wertz, A. L., and Macy, I. G.: Human Milk Studies: XX. The Diet of Lactating Women and the Collection and Preparation of Foods and Human Milk for Analysis, Am. J. Dis. Child., this issue, p. 142.

| | Ascorbic Acid Mg. per 100 cc. | |
|---|---|---|
| | Range | Average |
| Neuweiler, W.: Vitamin C Content of Human Milk, Ztschr. f. Vitaminforsch. **4:** 39-54, 1935; Nutrition Abstr. & Rev. **5:** 91, 1935. Studies using guinea pig and titration method | 4-7 | ... |
| Harris, L. J., and Ray, S. N.: Diagnosis of Vitamin C—Subnutrition by Urine Analysis, Lancet **1:** 71-77, 1935. 13 subjects. Observations on women in June and July, 2 to 6 months post partum. Indophenol titration | 2.7-8.5 | 5.6 |
| Selleg and King.[1] 17 subjects. Bessey and King method: | | |
| Value for 3d to 6th day | ..... | 5.3 |
| Value for 5th day | ..... | 5.6 |
| Value for 6th day | ..... | 5.8 |
| Value for 7th day | ..... | 6.0 |
| Value for 8th day | ..... | 6.6 |
| Value for 9th day | ..... | 6.4 |
| Value for 10th day | ..... | 6.4 |
| Wachholder, K.: Vitamin C Supply for Infants, Klin. Wchnschr. **15:** 593-596, 1936; Nutrition Abstr. & Rev. **6:** 471, 1936 | 4.2-5.6 | ... |
| Ferdinand, H.: The Vitamin C Content of Human and Cow's Milk in Spring, Klin. Wchnschr. **15:** 1311-1312, 1936; Nutrition Abstr. & Rev. **6:** 660, 1937. | | |
| Studies in winter. Methylene blue titration | ..... | 2.5 |
| Studies in spring | ..... | 1.3 |
| Stoerr, E.: Milk as a Source of Vitamin C, Human Milk, Cow's Milk, Raw and Heated, Seasonal Variation, Condensed and Dried Milk, Rev. franç. de pédiat. **12:** 427-438, 1936; Nutrition Abstr. & Rev. **6:** 660, 1937 | ..... | 3.0 |
| de Haas, J. H., and Meulemans, O.: The Ascorbic Acid Content of Human Milk in Batavia, Geneesk. tijdschr. v. Nederl.-Indië **76:** 2277-2296, 1936; Chem. Abstr. **31:** 443, 1937. | | |
| 500 observations on natives. Tillman's method. | ..... | 4.2 |
| 1,000 observations on Chinese | ..... | 3.8 |
| Studies of Europeans in Batavia | 6-7 | ... |
| Kasahara, M., and Kawashima, K.: Seasonal Variation in the Vitamin C Content of Human Milk, Ztschr. f. Kinderh. **58:** 191-192, 1936; Nutrition Abstr. & Rev. **6:** 660, 1936. Studies using Tillman's method | ..... | 4.5 |
| Gaehtgens, G., and Werner, E.: Administration of Vitamin C to Nursing Mothers, Arch. f. Gynäk. **165:** 63-75, 1937; Nutrition Abstr. & Rev. **7:** 1083, 1938. 10 subjects studied 4th to 11th day of lactation. Control group, no vitamin C added to diet. Tillman's method | 1.8-7.6 | ... |
| Correns, A. E.: The Vitamin C Content of Human Milk and Cow's Milk in Summer. Klin. Wchnschr. **16:** 81-83, 1937; Chem. Abstr. **31:** 3533, 1937. Studies at 4 months post partum. Tillman's method | 2.5-3.6 | ... |
| Widenbauer, F., and Kuhner, A.: Ascorbic Acid Studies on Lactating Women, Ztschr. f. Vitaminforsch. **6:** 50-75, 1937; Chem. Abstr. **31:** 2655, 1937 | 0.5-2.2 | ... |
| Bauman, T.: The Seasonal Variations in the Vitamin C Content of Human and Cow's Milk on the Infant's Requirement of Vitamin C and in the Adequacy of the Supply of Vitamin C of the Swiss Urban Population, Schweiz. med. Wchnschr. **67:** 962-965, 1937; Nutrition Abstr. & Rev. **7:** 1084, 1937. | | |
| 14 wet nurses studied in November-December | 1.0-1.7 | ... |
| 14 wetnurses studied in summer | Up to 3.2 | ... |
| Mothers studied in November-January | 2.5-3.0 | ... |
| Mothers studied in February-May | 3.5-5.0 | ... |
| Mothers studied in June-October | 4.0-7.5 | ... |
| Sinkko, E. I.: The Relation of Infant Mortality and Mortinatality to the Seasonal Variations in the Vitamin C Content of Breast Milk in Finland, Acta pædiat. **21:** 407-427, 1937; Chem. Abstr. **33:** 692, 1939. 10 subjects. 564 observations in February and September. Tillman's method. | | |
| February | ..... | 1.8 |
| September | ..... | 4.6 |
| Sadovsky, Weber and Wertheimer.[3] Determinations for 147 samples of milk from 62 mothers of Palestine during the first 8 days post partum. | | |
| 1st day | ..... | 2.63 |
| 2d day | ..... | 3.48 |
| 3d day | ..... | 5.41 |
| 4th day | ..... | 6.27 |
| 5th day | ..... | 5.55 |
| 6th day | ..... | 5.86 |
| 7th day | ..... | 5.48 |
| 8th day | ..... | 5.52 |

| | Ascorbic Acid Mg./per 100 cc. | |
|---|---|---|
| | Range | Average |
| Quesada, R.: The Vitamin C Content of Mother's Milk, Rev. Assoc. bioquim. argent. **5:** 24-26, 1940; Chem. Abstr. **36:** 140, 1942. 74 subjects at different stages of pregnancy, or at or after delivery | 2.1-9.0 | 5.1 |
| Winkler, H., and Heins, E.: Vitamin C Content of Human Milk in Summer and Winter, Ztschr. f. Geburtsh. u. Gynäk. **117:** 148-164, 1938; Dairy Sc. Abstr. **1:** 92-93, 1939; Chem. Abstr. **34:** 2887, 1940. Studies in late pregnancy and the first 10 days post partum. | | |
| Colostrum in winter | ..... | 2.5 |
| Colostrum in summer | ..... | 3.3 |
| "True milk," after 4th day in winter | ..... | 4.1 |
| "True milk," after 4th day in summer | ..... | 5.7 |
| Kroker, F.: Vitamin C in Cow's and Human Milk; Possibility of Producing a Vitamin C Milk, Milchwirtsch. Forsch. **19:** 318-338, 1938; Nutrition Abstr. & Rev. **8:** 652, 1939. Studies in first 10 days of lactation with 9 subjects. Indophenol titration: protein precipitated with ammonium sulfate | 2.2-4.2 | ... |
| Ingalls, Draper and Teel.[3] | | |
| One observation on each of 17 women, 4 to 5 days post partum, Indophenol titration after precipitation of protein with metaphosphoric acid | 1.7-7.2 | 4.4 |
| One observation on each of 15 women, 7 to 8 days post partum | 2.7-6.6 | 4.6 |
| One observation on each of 13 women 14 days post partum | 2.6-6.1 | 4.6 |
| Braestrup, P. W., and Lieck, H.: Biological Estimation of Vitamin C in Breast Milk, Hospitalstid. **81:** 913-916, 1938; Nutrition Abstr. & Rev. **8:** 652, 1939. Wetnurses on hospital diet without addition of fruit, in winter and spring. Indophenol titration after addition of 5 per cent metaphosphoric acid | 2.0-3.0 | ... |
| Cimmino, A.: Vitamin C in Human and Cow's Milk and in the Milk of Other Mammals, Quad. nutrizione **5:** 239-288, 1938; Nutrition Abstr. & Rev. **8:** 969, 1939. Studies of 5 women. Indophenol titration | ..... | 2.7 |
| Traversaro, J. C., and Quesada, R.: Vitamin C in Human Milk, Rev. Assoc. bioquim. argent. **3:** 7-9, 1938; Nutrition Abstr. & Rev. **8:** 1087, 1939. Studies of 6 subjects. | ..... | 5.0 |
| Chu, F. T.; Woo, T., and Sung, C.: A Study of Vitamin C Metabolism in Lactating Mothers, Chinese J. Physiol. **13:** 383-394, 1938; Nutrition Abstr. & Rev. **8:** 1086, 1939. Studies of 4 subjects on low vitamin C diet for 15 days. Indophenol titration | ..... | 4.0 |
| Gedda, E., and Kjellberg, K.: Vitamin C Content of the Breast Milk of Mothers in Gothenburg, Acta pædiat. **26:** 177-183, 1939; Chem. Abstr. **34:** 6331, 1940. Studies of 205 subjects in autumn. Direct titration with dichlorophenol-indophenol after addition of metaphosphoric acid | ..... | 4.2 |
| Studies of 216 subjects in spring | ..... | 3.6 |
| Studies of 421 subjects in autumn and spring | ..... | 3.9 |
| Burviana, L.: The Total Ascorbic Acid in the Milk of Some Mammals, Lait **19:** 449-454, 1939; Chem. Abstr. **33:** 7865, 1939. Studies using Martin and Bonsignore method | ..... | 3.0 |
| El-Gholmy, A. A.: The Ascorbic Acid Content of Human Milk and the State of Vitamin C Nutrition of Children Attending the Child Welfare Center, Thesis, Fouad I University, Cairo, Egypt; Nutrition Abstr. & Rev. **9:** 729, 1940. Studies of 140 subjects | 0.4-8.2 | 3.8 |
| Concepcion, I., and Gargaritano, M. L.: Studies on Vitamin C: III. The Vitamin C Content of Milk and Vegetable Extracts Available for Infants, J. Philippine Islands M. A. **19:** 1-6, 1939; Nutrition Abstr. & Rev. **9:** 93, 1939. Studies using indophenol titration | 4.0-5.0 | ... |
| Møller-Christensen, E., and Thorup, C.: The Vitamin C Content of Umbilical Cord Blood, Maternal Blood and Colostrum, Zentralbl. f. Gynäk. **64:** 1858-1861, 1940; Am. J. Obst. & Gynec. **43:** 585, 1942; Chem. Abstr. **36:** 3538, 1942. Colostrum studies with 10 subjects | ..... | 0.8 |
| Gurgoz, M.: Vitamin C Content of Cow Milk and Its Importance in Infant Feeding, Lait **20:** 279-289, 1940; Dairy Sc. Abstr. **2:** 134, 1940; Chem. Abstr. **35:** 3344, 1941 | ..... | 3.8 |

being filtered in order that clear filtrates might be obtained for those methods which require them.

The first chemical method for the determination of vitamin C became available in 1933. Workers have refined the technic employing dichlorophenolindophenol from several approaches. In obtaining the values presented in this paper, the Carruthers[4] method was used first. Later, Hochberg and co-workers[5] reported another modification of the dichlorophenolindophenol procedure, and Roe and Kuether[6] published a method using dinitrophenylhydrazine; these two methods, after investigations, also were used to determine values for vitamin C reported in this paper.

## RESULTS

*Oxidation of Ascorbic Acid.*—Forty-six twenty-four hour collections from the women receiving known diets and 22 four to eight hour collections from the women whose diets were self chosen were analyzed for ascorbic acid and total vitamin C (ascorbic acid plus dehydroascorbic acid) by the Hochberg method.[5] Nineteen of the twenty-four hour samples contained dehydroascorbic acid. In the 19 samples the ascorbic acid averaged 4.0 mg. and the dehydroascorbic acid 0.5 mg. per hundred cubic centimeters, the latter amounting to 11 per cent of the total vitamin C. Seven of the four to eight hour samples contained dehydroascorbic acid. In these the ascorbic acid averaged 2.1 mg. and the dehydroascorbic acid 0.6 mg. per hundred cubic centimeters, the latter amounting to 22 per cent of the total vitamin C. Although the two sets of samples contained amounts of dehydroascorbic acid which were approximately the same (0.5 and 0.6 mg. per hundred cubic centimeters), the average ascorbic acid content of the twenty-four hour milk samples was higher; therefore the dehydroascorbic acid accounted for a smaller percentage of the total vitamin C in the twenty-four hour collections than in the four to eight hour samples.

Previous workers have found that cow's milk expressed without exposure to light and air does not contain dehydroascorbic acid[7] and that the production of dehydroascorbic acid can be retarded by keeping the milk in a dark place and in an atmosphere of nitrogen.[7a] Since the findings for cow's milk are different from those for human milk presented here, several factors may have affected the oxidation of the human milk samples. During the first ten days post partum the expression of four hours' milk secretion required fifteen to ninety minutes. Expression of the other milk samples required as much as an hour's time. Therefore, there was an unavoidable delay before the metaphosphoric acid preservative could be added to any vitamin C aliquot and during this time there was some exposure to light and air. At the hospital the milk was expressed into sterile aluminum cups, but as soon as a few cubic centimeters had been collected the milk was poured into a glass graduate. Although metals cause oxidation of ascorbic acid, aluminum causes less oxidation of vitamin C than other metals. Further differences were that the 6 per cent metaphosphoric acid solution used with the twenty-four hour collections was not made with deaerated water, and the composite collection bottles[3] contained air. The four to eight hour samples reached the laboratory containing 12 per cent metaphosphoric acid, and many were in an atmosphere of nitrogen. All vitamin C aliquots were placed in amber bottles and kept in the refrigerator. These facts may have some bearing on the slightly larger percentage of twenty-four hour samples containing dehydroascorbic acid and, with the data, emphasize the importance of preventing oxidation as much as possible during the collection of milk samples for the determination of reduced ascorbic acid.

During the course of this study, the preservation of total vitamin C was studied in samples of milk, food and urine which had been stored from six to one hundred and twenty-two days at —25 C. in flasks containing nitrogen. The data obtained will be the subject of another paper.

*Variations in the Concentration of Ascorbic Acid at Four Hour Intervals Throughout the Day and Night.*—Thirty-seven individual obser-

4. Carruthers, C.: An Improved Photometric Method for Ascorbic Acid, Indust. & Engin. Chem. (Anal. Ed.) **14**:826-828 (Oct. 15) 1942.

5. Hochberg, M.; Melnick, D., and Oser, B. L.: Photometric Determination of Reduced and Total Ascorbic Acid, Indust. & Engin. Chem. (Anal. Ed.) **15**:182-188 (March 15) 1943.

6. Roe, J. H., and Kuether, C. A.: The Determination of Ascorbic Acid in Whole Blood and Urine Through the 2, 4 Dinitrophenylhydrazine Derivative of Dehydroascorbic Acid, J. Biol. Chem. **147**:399-407 (Feb.) 1943.

7. Hand, D. B.: Reduced and Total Vitamin C in Milk, J. Dairy Sc. **26**:7-12 (Feb.) 1943. Kon, S. K. and Watson, M. B.: XXVII. The Vitamin C Content of Cow's Milk, Biochem. J. **31**:223-226, 1937.

7a. Woessner, W. W.; Weckel, K. G., and Schuett H. A.: The Effect of Commercial Practices on Vitamin C and Dehydroascorbic Acid in Milk, J. Dairy Sc. **23**: 1131-1141 (Aug.) 1940. Holmberg, C. G.: The Effect of Pasteurizing on the Vitamin C Content of Winter Milk, Kgl. Fysiograf. Sällsk. Lunk Förh. **7**: 105-110, 1937.

ations were made by the Carruthers method [4] n the concentration of reduced ascorbic acid in uman milk when this was expressed at four our intervals throughout the day and night. our consecutive twenty-four hour collections ere obtained from 1 woman forty-five to forty-ight days after the birth of her infant and a one ay collection fifty-nine days after the infant's irth. Each collection was made at a four hour nterval except that at 2 a.m., in her home, hile she continued her usual activities as a ousewife. Two hospitalized obstetric patients ere studied for one day nine and eight days ost partum, respectively. The values are plotted n chart 1.

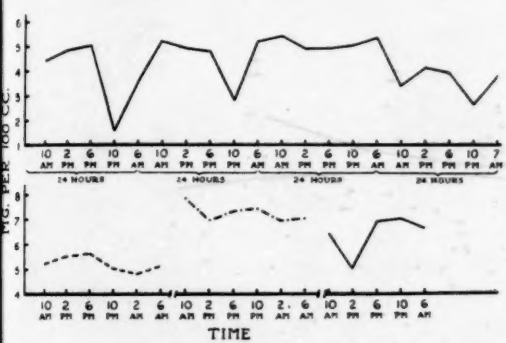



Chart 1.—Concentrations of vitamin C in human milk four hour intervals. Top: Vitamin C in the milk of a oman studied during four consecutive days, forty-five forty-eight days post partum. Lower left: Vitamin C the milk of a woman studied nine days post partum. ower center: Vitamin C in the milk of a woman udied eight days post partum. Lower right: Vitamin in the milk of a woman studied fifty-nine days post rtum.

The lowest concentration of ascorbic acid did ot always occur at the same time of day, but in e samples analyzed it seemed to occur most equently at 10 p. m. or 2 a. m. (chart 1). here was less variation in the values of the milk om the 2 patients who were in bed a large part the day and whose intakes of vitamin C were w. The greatest daily variation in ascorbic id concentration was shown by the milk of the tient who was studied for four days, and the eatest range in any one day was from 5.0 mg. 1.6 mg. per hundred cubic centimeters, a crease of 3.4 mg. per hundred cubic centi-eters.

From the variation in concentration of ascorbic id it is evident that analysis of a single sample es not provide an accurate measure of the tamin C content of human milk during twenty-ur hours. Accordingly, a random sample of milk may not give an accurate figure for estimating the amount of vitamin C received by an infant during a twenty-four hour period.

*Variation in the Vitamin C Content of Human Milk During Lactation.*—Selleg and King [1] have reported an average value of 5.3 mg. reduced ascorbic acid in human milk for the third to sixth day post partum and average values of 5.6, 5.8, 6.0, 6.6, 6.4, 6.4 mg. per hundred cubic centimeters of milk for the fifth through the tenth day post partum. Ingalls, and co-workers [2] found the average values of reduced ascorbic acid per hundred cubic centimeters of milk for 17 women to be: four to five days post partum, 4.4 mg.; seven to eight days, 4.6 mg.; fourteenth day, 4.6 mg. Sadovsky, Weber and Wertheimer,[8] in 147 milk samples obtained from 62 mothers of Palestine during the first eight days post partum, found the average ascorbic acid contents to be, respectively: 2.6, 3.5, 5.4, 6.3, 5.6, 5.9, 5.5 and 5.5 mg. per hundred cubic centimeters.

In the present study the average total vitamin C values [6] for the twenty-four hour collections of milk obtained from 4 women during the first ten days post partum were, respectively, 5.1, 7.5, 7.9, 7.5, 7.2, 6.8, 7.0, 7.2, 7.5 and 7.0 mg. per

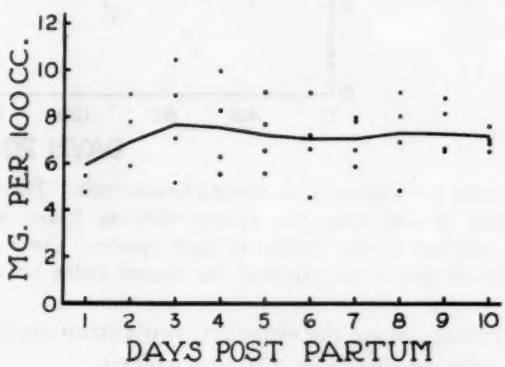



Chart 2.—Vitamin C in immature human milk from the women receiving the known diet (twenty-four hour collections). The curve was obtained by three point smoothing of the means.

hundred cubic centimeters. These values represent 37 of the 60 collections of milk obtained from women receiving diets which were analyzed. These averages per day show that the highest concentration of vitamin C occurred about the third day. The smoothed mean curve shown in chart 2 rises during the first three days, then levels off at approximately 7 mg. per hundred

8. Sadovsky, A.; Weber, D., and Wertheimer, E.: The Concentration of Vitamin C in Blood During and After Pregnancy, J. Lab. & Clin. Med. **25**:120-131 (Nov.) 1939.

cubic centimeters from the third through the tenth day.

For the milk from the women studied during the first ten days post partum the "analysis of variance" technic did not show significant differences among the values for the same mother's milk of different days. Similarly, with the milk from women studied during five day periods six, eight and ten months post partum significant differences among the values for different days were not shown, but there was a significant difference among the vitmain C concentrations of the milk samples from different women.

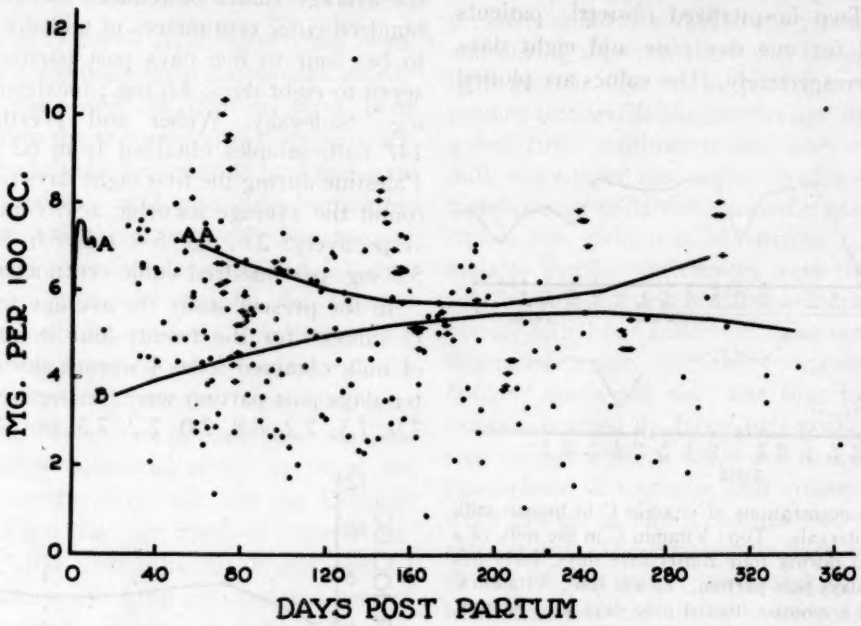



Chart 3.—Vitamin C in mature human milk. The values for the vitamin C contents in twenty-four hour collections of milk from the women receiving known diets are shown by the bisected points, for which curve *AA* was obtained by the method of least squares. Curve *A* is repeated from chart 2. The points showing values of the vitamin C in milk from the women eating self-chosen diets are represented by curve *B*.

Sadovsky and associates [8] found a rather marked and progressive rise in the concentration of reduced ascorbic acid in human milk until the fourth day post partum and lower values thereafter. Selleg and King [1] observed a rise in the concentration from the fifth through the tenth day post partum and attributed this to the improvement of the dietary in the hospital over that being eaten before admission.

Chart 3 shows the results of analyses for total vitamin C [6] in 65 of the 90 twenty-four hour collections of milk obtained sixty-eight to three hundred and six days post partum from the women receiving known diets and on 166 of the 179 four to eight hour samples from the women eating self-chosen diets. The best-fitting curve for the data from each group of women indicates the trend of the concentration of total vitamin C as lactation progresses. For the women receiving known diets the maximum concentration on the curve is 7.0 mg. and the minimum 5.7 mg. per hundred cubic centimeters of milk; the curve for the samples from women eating self-chosen diets shows a maximum concentration of 5.5 mg. and a minimum of 3.6 mg. per hundred cubic centimeters. The curve for the data from analyses of twenty-four hour collections obtained during the first ten days post partum (chart 2) also is shown on chart 3, so that the levels of vitamin C in colostral and mature milk may be studied.

## SUMMARY

Determinations of vitamin C were made on 329 collections of immature human milk obtained during the first ten days post partum and mature milk obtained as late as the twelfth month of lactation.

The oxidized form of ascorbic acid, dehydroascorbic acid, was found in over 35 per cent of the 68 milk samples analyzed and accounted for 14 per cent of the total vitamin C (ascorbic acid plus dehydroascorbic acid) in those samples.

Complete collections of milk made at four hour ervals throughout the day and night were nd to vary in their ascorbic acid contents. us, accurate values for the daily secretion of s vitamin in breast milk can be obtained only analysis of twenty-four hour collections.

Total vitamin C or ascorbic acid, or both, re determined on 60 twenty-four hour collec- ns of milk secreted during the first ten days st partum and on 90 twenty-four hour collec- ns obtained from 10 women during periods of five consecutive days at intervals in lactation. Likewise, analyses for total vitamin C and for ascorbic acid, or for total vitamin C only, were made on 179 samples of mature milk from 66 women, each sample representing the secretion during four to eight hours while the women were eating diets of their own choice.

The average total vitamin C for milk collected during the first ten days post partum was 7.2 mg. per hundred cubic centimeters, and that for partial and complete daily collections of mature milk was 5.2 mg. per hundred cubic centimeters.

# HUMAN MILK STUDIES

## XXVI. VITAMIN A AND CAROTENOID CONTENTS OF COLOSTRUM AND MATURE HUMAN MILK

MARJORIE LESHER, M.S.; JAMES K. BRODY, M.S.; HAROLD H. WILLIAMS, PH.D., AND ICIE G. MACY, PH.D.

DETROIT

The vitamin A and carotenoid contents of human milk have been of interest for a number of years. Quantitative estimations of the vitamin A in breast milk were carried out as early as 1923 by Kennedy, Palmer and Schlutz.[1] These investigators pointed out the superiority of human milk over cow's milk in vitamin A and also indicated the effect of a very poor diet on mother's milk. Macy and her associates reported, in addition to quantitative estimations of vitamin A,[2] the differences between cow's milk and human milk,[3] the variations in composition of milk among individual women [4] and the effect of vitamin supplementation of the diet.[5] Debré and Busson,[6] using a biologic method, compared human milk and the various forms of cow's milk used in infant feeding in France.

Vitamin A is a stimulus for the formation of new cells and as such is an important factor in growth and in the maintenance of tissues. Deficiency of vitamin A may produce atrophy of epithelium, followed by formation of stratified, keratinized epithelial tissue, from which arise numerous pathologic conditions. Bessey and Wolbach [7] stated that the trachea and the bronchi are the most common sites and the points of the earliest occurrence of "keratinizing metaplasia" in human infants. Thus, an adequate supply of vitamin A is of particular importance as a protection against respiratory infections in the young. Indeed, Blegvad,[8] in following the histories of Danish infants who had ocular afflictions from deficiency of vitamin A, reported that in a large proportion of the fatal cases death was due to respiratory disease. Likewise, the prevalence of respiratory complications in vitamin A deficiency was brought out among the 13 cases studied by Blackfan and Wolbach,[9] among the 13 cases of Thalberg [10] and among the 203 cases of Sweet and K'ang.[11] Severe vitamin A deficiency results in xerophthalmia. As a component of the visual purple, vitamin A plays a vital role in the visual processes, and even slight deficiencies may cause detectable night blindness.

Breast milk has been shown to contain larger amounts of vitamin A than does cow's milk.

From the Research Laboratory of the Children's Fund of Michigan.

1. Kennedy, C.; Palmer, L. S., and Schlutz, F. W.: The Vitamin Content of Breast Milk, Tr. Am. Pediat. Soc. **35**:26-33, 1923.

2. Macy, I. G.; Outhouse, J.; Graham, A., and Long, M. L.: Human Milk Studies: II. The Quantitative Estimation of Vitamin A, J. Biol. Chem. **73**: 175-188 (May) 1927.

3. Outhouse, J.; Macy, I. G.; Brekke, V., and Graham, A.: Human Milk Studies: IV. A Note on the Vitamin A and B Content of Cow's Milk, J. Biol. Chem. **73**:203-208 (May) 1927.

4. Macy, I. G.; Outhouse, J., and Hunscher, H.: The Variability in Vitamin Content of Human Milks, J. Home Econ. **20**:897-900 (Dec.) 1928.

5. McCosh, S. S.; Macy, I. G.; Hunscher, H. A.; Erickson, B. N., and Donelson, E.: Human Milk Studies: XIII. Vitamin Potency as Influenced by Supplementing the Maternal Diet with Vitamin A, J. Nutrition **7**:331-336 (March) 1934.

6. Debré, R., and Busson, A.: Teneur en vitamine A des différents laits ou de certains dérivés du lait, Compt. rend. Soc. de biol. **114**:1297-1299, 1933.

7. Bessey, O. A., and Wolbach, S. B.: Vitamin A Physiology and Pathology, J. A. M. A. **110**:2072-20 (June 18) 1938.

8. Blegvad, O.: Xerophthalmia, Keratomalacia a Xerosis Conjunctivae, Am. J. Ophth. **7**:89-117 (Fe 1924.

9. Blackfan, K. D., and Wolbach, S. B.: Vitamin Deficiency in Infants, J. Pediat. **3**:679-706 (Nov.) 19

10. Thalberg, J.: Zur Casuistik der durch Ina itionszustände bedingten Hornhautgangrän, Arch. Augenh. **12**:315-332, 1883.

11. Sweet, L. K., and K'ang, H. J.: Clinical a Anatomic Study of Avitaminosis Among the Chine Am. J. Dis. Child. **50**:699-734 (Sept.) 1935.

12. (*a*) de Haas, J. H., and Meulemans, O.: Caroten and Vitamin A Contents of Cows' Milk and of Cow Milk Mixtures: A Contribution to Vitamin A Prophylaxis, Ztschr. f. Vitaminforsch. **7**:1-10, 1938. (*b*) Ch vallier, A.; Giraud, P., and Dinard, C.: Recherch sur la teneur en vitamine A de différents laits destin a l'alimentation des nourrissons dans la région ma seillaise, Nourrisson **28**:45-67 (March) 1940. Kenne and others.[1] Macy and others.[2] Outhouse and others

However, vitamin A deficiency has been reported in breast-fed infants. In 1883 Thalberg,[10] in Russia, reported the occurrence of xerophthalmia in sucklings whose mothers were in a poor nutritional state. De Haas and Meulemans[13] found in a study of infants with xerophthalmia, in Batavia, that 3 of 11 were breast fed. Analyses of the milk of the 3 mothers revealed no vitamin A in the milk of 2 and only a very small amount in that of the third. Thatcher and Sure[14] reported the occurrence of xerophthalmia and ultimate death of a suckling of a mother who had a diet low in vitamin A. One of the patients of Blackfan and Wolbach[9] had been breast fed.

Although these cases of extreme deficiency may be rare, Stiebeling[15] has shown that in 1936 only one fifth of the families in this country ate diets which met the recommendations of the Food and Nutrition Board of the National Research Council. Other dietary surveys carried out on special groups by Stiebeling and Phipard[16] and Wiehl,[17] as well as surveys of vitamin A deficiency (determined by the dark adaptation test) by Jeans and Zentmire[18] and Getz, Hildebrand and Finn,[19] have indicated that the problem is significant. Especially is there need for a more comprehensive study of the vitamin A and carotenoid contents of mother's milk during the progression of lactation when the diet of the mother is controlled in quality and known in quantity and the milk is quantitatively collected under comparable conditions.

13. de Haas, J. H., and Meulemans, O.: Vitamin A and Carotenoids in Blood: Deficiencies in Children Suffering from Xerophthalmia, Lancet **1**: 1110-1111 (May 14) 1938.

14. Thatcher, H. S., and Sure, B.: Avitaminosis: II. Pathologic Changes in Tissues of the Albino Rat during Early Stages of Vitamin A Deficiency, Arch. Path. **13**:756-765 (May) 1932.

15. Stiebeling, H. K.: How Well Fed Is the American Family? The Food Front, Office of Defense Health and Welfare Services, Federal Security Agency, 1942, p. 18-21.

16. Stiebeling, H. K., and Phipard, E. F.: Diets of Families of Employed Wage Earners and Clerical Workers in Cities, Circular 507, United States Department of Agriculture, 1939.

17. Wiehl, D. G.: Medical Evaluation of Nutritional Status: VII. Diets of High School Students of Low Income Families in New York City, Milbank Mem. Fund Quart. **20**:61-82 (Jan.) 1942; Diets of a Group of Aircraft Workers in Southern California, ibid. **20**:329-336 (Oct.) 1942.

18. Jeans, P. C., and Zentmire, Z.: The Prevalence of Vitamin A Deficiency Among Iowa Children, J. A. M. A. **106**:996-997 (March 21) 1936.

19. Getz, H. R.; Hildebrand, G. B., and Finn, M.: Vitamin A Deficiency in Normal and Tuberculous Persons, J. A. M. A. **112**:1308-1310 (April 8) 1939.

## EXPERIMENTAL STUDY

If one is to determine accurately the content of vitamin A and that of carotenoids in mother's milk, collection of all the milk is of extreme importance, for the concentration in the first milk expressed from the breast is considerably different from that in the last portion.[20] The technics employed in the present study[21] insured complete stripping of the breasts. The study is unique in that 50 twenty-four hour collections of milk were obtained from women during the first ten days post partum and 76 twenty-four hour collections were obtained at various intervals in the lactation period while the women were ingesting diets of equal quality and of known composition. In addition, 188 samples representing four to eight hours' production of milk were obtained from 69 women whose diets were self chosen. The details of the general plan of the investigations, the selection of subjects and the collection and preparation of samples are given in a preceding paper.[22]

### METHOD

Most of the current methods of analyzing milk for vitamin A involve saponification and subsequent extraction, and washing, drying and adjusting of the volume of the extract. In this study the saponification was eliminated by direct extraction of the sample. Variations in the influences of the fatty constituents of the extract, which usually are partially removed by saponification, were eliminated by use of the increment method, in which the vitamin A standard is subjected to the same effects in the antimony trichloride reaction as is the vitamin A in the sample. Thus, the manipulations of saponification and of washing, drying and adjusting of the volume of the extract, as well as the possible errors arising from this additional handling, were avoided. Experience indicates that vitamin A was stable in the extract not only during the time required for analysis but for periods of at least seven hours. The material directly extracted apparently contains substances which exert a stabilizing effect on the vitamin A.

*Procedure of Extraction.*—The procedure was based on the methods of Rose and of Gottlieb for the extraction of fat in milk.[23]

20. (*a*) Chevallier, A.; Giraud, P., and Dinard, C.: Sur la teneur du lait de femme en vitamine A, Compt. rend. Soc. de biol. **131**:373-375, 1939; footnote 12 *b*. (*b*) Neuweiler, W.: Der Vitamin A- und Carotingehalt der Frauenmilch, Ztschr. f. Vitaminforsch. **4**:259-271, 1935.

21. Davies, V.: Human Milk Studies: XXI. A Simple Technic for the Manual Expression of Mother's Milk, Am. J. Dis. Child., this issue, p. 148.

22. Macy, I. G.; Williams, H. H.; Pratt, J. P., and Hamil, B. M.: Human Milk Studies: XIX. Implications of Breast Feeding and Their Investigation, Am. J. Dis. Child., this issue, p. 135.

23. Rose (1888) and Gottlieb (1892), cited by Woodman, A. G.: Food Analysis, ed. 2, New York, McGraw-Hill Book Company, Inc., 1924, p. 115.

To 3 samples of milk of 10 cc. each [24] in 50 cc. glass-stoppered centrifuge tubes were added: first, 2 cc. of concentrated ammonium hydroxide and then 10 cc. of 95 per cent ethyl alcohol. Fifteen cubic centimeters of the extracting solvent (1 part ether and 3 parts purified benzine U. S. P.) was added, each tube was stoppered and a small amount of paraffin oil was spread around the top of the stopper. The levels of the liquid before and after the addition of the ether-benzine mixture were marked on the tube as a check on the complete recovery of the ether layer. This recovery was complete when the ratio of ether to purified benzine and the amounts of water and alcohol indicated were used.

The sample was extracted by shaking the tube with end over end inversion for five minutes. The tubes were centrifuged twice, each time for five minutes. After the first centrifuging, the ether-benzine extract was not homogeneous; so the tube was shaken very gently to mix the ether-benzine layer without disturbing the precipitate any more than was necessary before the sample again was centrifuged. Twelve to 13 cc. of the ether-benzine extract was drawn off by gentle suction, cautiously, so that none of the alcohol-water layer or the precipitate between the layers would get into the ether-benzine extract.

*Determination of Carotenoids and Vitamin A.*—Ten cubic centimeters of extract from each sample was measured into each of three optically matched test tubes. The carotenoid determination was made from the transmission reading against a solvent blank at 440 millimicrons on a Coleman Universal Spectrophotometer.

The increment method employed in the determination of vitamin A has been used by several investigators [25] and is recommended specifically for vitamin A by Oser, Melnick and Pader.[26] The ether-benzine solvent was evaporated from the samples in the test tubes by warming in a water bath at a temperature not over 50 C. and bubbling nitrogen through the solution. Wintrobe pipets were used for delivering the nitrogen because their small capillaries and surface areas could easily be washed free of oily residue. Each residue was dissolved in 1 cc. of chloroform. To one of the tubes 0.1 cc. of a vitamin A standard in chloroform solution was added, to another tube 0.2 cc. and to the third tube no standard was added.

The spectrophotometer was set at 620 millimicro and the galvanometer adjusted to read directly in cent transmission against a solvent blank of 1 cc. chloroform and 9 cc. of antimony trichloride reage The Carr-Price color reaction [27] was carried out the addition of 9 cc. of antimony trichloride reage to the sample tube which was in position in the spectr photometer. The reagent was delivered in two secon by blowing from a wide-tipped pipet, thorough mixi being accomplished with the delivery. The galvano eter was read exactly eight seconds after the beginni of the addition of the antimony trichloride. Tests wi 1 cc. of an aqueous solution of cupric chloride a 9 cc. of water indicated that this method of deliver gave thorough mixing in the cell. Likewise, it w shown that eight seconds was the maximum length time required for the galvanometer to come to re with a stable solution (aqueous cupric chloride) treat like the vitamin A samples.

Occasionally a sample produced a cloudy reacti with antimony trichloride. This seemed to be cha acteristic of the milk, and repeated samples would gi the same result. In these cases the samples were sapon fied and the vitamin A determined by a modificatio of Dann's procedure.[28]

*Preparation of Reagents.*—Ether, chemically pur was distilled and kept in a brown bottle in the refri erator. It was used until it gave a positive reactio when tested for peroxides. If the formation of pe oxides was only slight, these could be removed b shaking the ether with activated magnesia (Micr Brand no. 2641).

Chemically pure benzine (distilled at 35 to 65 C.) w dried over concentrated sulfuric acid and redistill shortly before use. The fraction distilled at 38 48 C. was collected.

Chloroform, chemically pure, was washed seven tim with equal volumes of water and was dried over anh drous potassium carbonate. It was then distilled, t first and the last 10 per cent being discarded. W storage in brown bottles in the refrigerator, the chlor form would remain stable for one to three days. was tested for oxidants each day. Small amounts oxidants could be removed by shaking with activat magnesia.

A saturated solution was prepared by dissolvi antimony trichloride, chemically pure, in freshly distill chloroform. The reagent was filtered and kept in brown bottle, which was stored in a refrigerator wh the reagent was not in use. Tests indicated that t color development with vitamin A was not affected b the concentration of the antimony trichloride reage in the range between a saturated solution and a soluti of half that concentration. Likewise, the color develo ment did not vary with temperatures from 15 to 35

*Preparation of Standards.*—(*a*) Carotenoids: A stan ard curve (log transmission vs. concentration) was pr pared by using crystalline beta carotene dissolved purified benzine (distilled at 38 to 48 C.). All readin were taken at 440 millimicrons.

24. For determinations on colostrum smaller samples were used and enough water added to make 10 cc.

25. Lamb, F. W.: Direct Quantitative Determination of Nicotinamide in Vitamin Mixtures, Indust. & Engin. Chem. (Anal. Ed.) **15**:352-355 (May 15) 1943. Harris, L. J., and Raymond, W. D.: CCL. Assessment of the Level of Nutrition: A Method for the Estimation of Nicotinic Acid in Urine, Biochem. J. **33**:2037-2051, 1939. Andrews, J. S.: Collaborative Study of Riboflavin Assay Methods, Cereal Chem. **20**:3-23 (Jan.) 1943. Melnick, D.: Collaborative Study of the Applicability of Microbiological and Chemical Methods to the Determination of Niacin (Nicotinic Acid) in Cereal Products, ibid. **19**:553-567 (Sept.) 1942. Hodson, A. Z., and Norris, L. C.: Fluorometric Method for Determining the Riboflavin Content of Foodstuffs, J. Biol. Chem. **131**:621-630 (Dec.) 1939.

26. Oser, B. L.; Melnick, D., and Pader, M.: Estimation of Vitamin A in Food Products, Indust. & Engin. Chem. (Anal Ed.) **15**:724-729 (Dec. 15) 1943.

27. Carr, F. H., and Price, E. A.: LXIV. Colo Reactions Attributed to Vitamin A, Biochem. J. 497-501, 1926.

28. Dann, W. J.: CCLXXI. The Transmission Vitamin A from Parents to Young in Mammals: The Carotene and Vitamin A Content of Cow's Colo trum, Biochem. J. **27**:1998-2005, 1933.

(*b*) Vitamin A: Distillation Products, Inc., concentrate of natural vitamin A ester, controls 4673 and 6617 (PC-2), was used as a standard. The E values, $E\ \frac{1\ \text{per cent}}{1\ \text{cm.}}$ 328 millimicrons),[29] were 106.00 and 90.2 respectively, for the controls 4673 and 6617 (PC-2) as measured in ethyl alcohol solution with the Beckman Spectrophotometer by the manufacturer. The value of crystalline vitamin A alcohol was taken as 1780 in calculating the potency of the concentrate on the basis of its absorption of ultraviolet rays. The antimony trichloride color as measured by the manufacturer on a Hardy Recording Spectrophotometer gave and E value (620 millimicrons) of 290 for control 4673. The amount of vitamin A in the concentrate was calculated on the basis of an E value of 4800 for crystalline vitamin A.

A chloroform solution of vitamin A with approximately 15 micrograms of the vitamin per cubic centimeter was prepared for the increment to be added to the samples. The concentration of this standard was checked by the antimony trichloride reaction with 0.1 cc. and 0.2 cc. aliquots of the solution and chloroform to make 1 cc. The transmission readings were referred to a standard curve (log transmission vs. concentration) made from one of the ampules of control 4673 and checked later with another ampule of the same lot. This standard chloroform solution of vitamin A was stable for about three days when kept in a refrigerator. However, its concentration was determined every day and sometimes twice a day if some was used both in the morning and in the late afternoon.

*Calculations.*—(*a*) Carotenoids: The concentration of carotenoids in the extract was read as beta carotene from the standard curve (log transmission vs. concentration).

(*b*) Vitamin A: The color produced by the reaction of carotenoids with antimony trichloride was slight, but a correction was applied for it in the determination of vitamin A. Oser, Melnick and Pader[26] showed that the reactions of antimony trichloride with vitamin A and carotene in the same solution are additive. The correction for carotenoids was based on this principle. A curve (density vs. micrograms of beta carotene) was prepared by measuring the color given at 620 millimicrons by antimony trichloride reacting with various amounts of beta carotene. From the amount of yellow pigment in the sample of extract the density ($\log \frac{100}{T}$, T = per cent transmission) owing to the carotenoids could be determined as if these were all beta carotene. Inasmuch as they were not, the correction was only approximate. After each of the transmission readings of the samples reacting with antimony trichloride was converted to density by the formula just given, the density due to the carotenoids could be subtracted. Another correction was applied to take care of the difference in the volumes of the reacting solutions owing to the addition of the vitamin A standard in the increment procedure. Then the corrected densities for each sample were plotted, drawn with density as the ordinate and micrograms of vitamin A added to the cell as the abscissa. On the left of the zero point on the abscissa the number of micrograms of vitamin A present in the cell before any standard was added was measured from right to left. A straight line was drawn through the three points representing 0.0, 0.1 and 0.2 cc. of vitamin A standard added. The point at which the line intercepted the axis (at zero density) gave the micrograms of vitamin A present in the amount of extract used in the cell.

Since the proper factor to be used for the conversion of vtamin A from micrograms to U. S. P. units is still unknown, the results of this investigation are reported in terms of the weight unit. However, since many persons are still accustomed to the biologic unit, U. S. P. units are given as determined with the factor 4.3 (1 microgram = 4.3 U. S. P. units). This is based on the results of Distillation Products, Inc.; the biologic activity of their crystalline vitamin A alcohol was estimated to be 4,300,000 U. S. P. units per gram. They state that this value is only approximate, and they do not present it as a conversion factor, but at present it seems the most logical figure to use.

*A Sample Calculation.*—Each of 3 10 cc. samples of milk was extracted with 15 cc. of the ether-benzine mixture. Ten cubic centimeters of each extract was used for the Carr-Price reaction.

The galvanometer readings (per cent transmission) at 440 millimicrons were 89.4, 89.4 and 89.0.

Referring these readings to the standard beta carotene curve, one finds a concentration of 0.18 microgram per cubic centimeter for the carotenoids in the extract.

0.18 microgram × 15 cc. = 2.7 micrograms in 10 cc. of milk.

2.7 micrograms × 10 cc. = 27 micrograms per hundred cubic centimeters of milk.

The following calculations are performed in the determination of vitamin A:

| | | | |
|---|---|---|---|
| Concentration of vitamin A in standard (micrograms per cubic centimeter) | 14.0 | ..... | ..... |
| Cubic centimeters of standard added to sample | ..... | 0.10 | 0.20 |
| Micrograms of vitamin A added to sample | ..... | 1.40 | 2.80 |
| Galvanometer reading (per cent transmission) | 63.8 | 54.8 | 47.0 |
| Density | 0.195 | 0.261 | 0.328 |
| Density corrected for volume | 0.195 | 0.264 | 0.335 |
| Micrograms of carotenoids in sample | 1.8 | 1.8 | 1.8 |
| Density due to reaction of carotenoids with antimony trichloride (as read from curve) | 0.013 | 0.013 | 0.013 |
| Density corrected for carotenoids | 0.182 | 0.251 | 0.322 |

The corrected densities are plotted against the amount of vitamin A added to the sample and the curve is drawn (chart 1) and extrapolated to give a value of 3.70 micrograms of vitamin A in 10 cc. of the milk extract. This is multiplied by 15/10 and by 10 to give 55.5 micrograms (rounded to 56) per hundred cubic centimeters of milk.

*Accuracy.*—To check the complete removal of vitamin A and carotenoids from the water-alcohol layer in a single extraction, most of the water-alcohol layer was removed and reextracted with the ether-benzine mixture. More of vitamin A or carotenoids was not obtained from any sample, indicating that the original extraction was complete. The method was estimated

29. $E\ \frac{1\ \text{per cent}}{1\ \text{cm.}}$ (328 millimicrons) is the extinction, or absorption, produced by a 1 per cent solution of the concentrate in a 1 cm. cell read at 328 millimicrons.

is calculated as: $\frac{\log \frac{I_o}{I}}{d \times \%\ \text{conc.}}$ in which $\log \frac{I_o}{I}$ equals

ical density, *d* equals length of light path through cell and "per cent concentration" equals grams of multiplied by 100, divided by cubic centimeters of vent.

to give within ± 10 per cent the total amount of vitamin A and carotenoids present.

To investigate the precision of the method, series of samples of cow's milk (four determinations in a series) were analyzed and the results examined for variation between different analyses of the same milk. Two representative series are shown in table 1. As would be expected, the determination of the carotenoids is more precise than that of the vitamin A.

For comparisons of the results obtained by the direct extraction method with those obtained by saponification, cow's milk (sometimes with cream added to give a vitamin A concentration more nearly like that of human milk) was analyzed by direct extraction and by the saponification methods of Dann[28] and Koehn,[30]

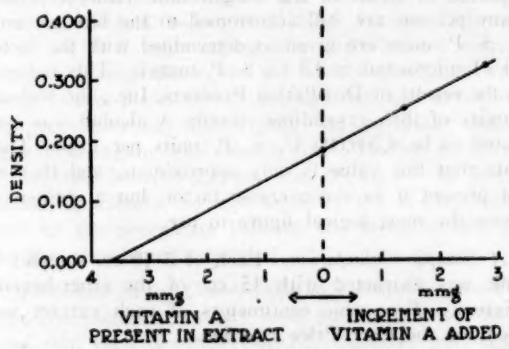



Chart 1.—Curve of the values obtained in the sample calculation by the increment method. The amount of vitamin A is represented by the point of intersection of the straight line curve with the abscissa (horizontal axis).

TABLE 1.—*Precision of Method*

| Series | | Carotenoids: Micrograms per 100 Cc. | Carotenoids: Deviation from Mean, per Cent | Vitamin A: Micrograms per 100 Cc. | Vitamin A: Deviation from Mean, per Cent |
|---|---|---|---|---|---|
| I | 1..... | 51 | +1.0 | 29 | +0.7 |
| | 2..... | 51 | +1.0 | 28 | −2.8 |
| | 3..... | 50 | −1.0 | 30 | +4.2 |
| | 4..... | 50 | −1.0 | 28 | −2.8 |
| | Mean..... | 50.5 | | 28.8 | |
| II | 1..... | 36 | 0.0 | 33 | −5.2 |
| | 2..... | 36 | 0.0 | 35 | +0.6 |
| | 3..... | 36 | 0.0 | 34 | −2.3 |
| | 4..... | 36 | 0.0 | 37 | +6.3 |
| | Mean..... | 36 | | 34.8 | |

somewhat modified. The increment procedure was used with all methods, since it has been observed that saponification did not always remove all of the substances which affected the color intensity of the antimony trichloride reaction. The results shown in table 2 indicate a suitable check between the methods considered from the point of view of accuracy and precision. The saponified samples (table 2) were extracted with ether. It was noted in earlier experiments when purified benzine U. S. P. was used with saponification that carotenoids were not completely extracted, although vitamin A apparently was.

30. Koehn, C. J.: Vitamin A Activity of Milk as Related to Pasture and Feeding Practices in Alabama, J. Dairy Sc. **26**:673-681 (Aug.) 1943.

## RESULTS

The concentrations of vitamin A and carotenoids found in human milk are presented in table 3. Averages, ranges and standard deviations are given for the known diet group and for the self-chosen diet group.[22] The averages for the vitamin A of the mature milk of the two groups are essentially the same (62 and 67 micrograms per hundred cubic centimeters, 267 and 258 U. S. P. units per hundred cubic centimeters) and also those for carotenoids (25 and 25 micrograms per hundred cubic centimeters). Average concentrations are given likewise for the immature milk of each of the first ten days post partum. No effort has been made to combine vitamin A and carotenoid values to give total vitamin A activity, for the vitamin A activity of the carotenoids in the individual samples is not known definitely.

TABLE 2.—*Comparison of Methods*

| Sample | Method | Carotenoids: Micrograms per 100 Cc. | Carotenoids: Result Obtained by Direct Extraction, per Cent | Vitamin A: Micrograms per 100 Cc. | Vitamin A: Result Obtained by Direct Extraction, per Cent |
|---|---|---|---|---|---|
| A | Direct extraction | 41 | ... | 31 | ... |
| | Dann..... | 42 | 102 | 35 | 113 |
| | Koehn..... | 42 | 102 | 27 | 87 |
| B | Direct extraction | 71 | ... | 39 | ... |
| | Dann..... | 70 | 98 | 43 | 110 |
| | Koehn..... | 62 | 87 | 43 | 110 |
| C | Direct extraction | 39 | ... | 36 | ... |
| | Dann..... | 40 | 102 | 34 | 94 |
| D | Direct extraction | 90 | ... | 53 | ... |
| | Koehn..... | 87 | 97 | 49 | 92 |
| E | Direct extraction | 101 | ... | 56 | ... |
| | Koehn..... | 96 | 95 | 55 | 98 |

TABLE 3.—*Vitamin A and Carotenoids Contents of Human Milk*

| Days Post Partum | Vitamin A: Subjects | Vitamin A: Samples* | Vitamin A: Mean, Micrograms per 100 Cc. | Vitamin A: Range, Micrograms per 100 Cc. | Carotenoids: Subjects | Carotenoids: Samples* | Carotenoids: Mean, Micrograms per 100 Cc. | Carotenoids: Range, Micrograms per 100 Cc. |
|---|---|---|---|---|---|---|---|---|
| 1 | 4 | 4 | 142 | 75-198 | 4 | 4 | 241 | 86-3 |
| 2 | 5 | 5 | 182 | 105-295 | 5 | 5 | 151 | 108-3 |
| 3 | 5 | 5 | 198 | 124-305 | 5 | 5 | 154 | 88-2 |
| 4 | 5 | 5 | 148 | 84-258 | 5 | 5 | 97 | 72-1 |
| 5 | 5 | 5 | 132 | 76-214 | 5 | 5 | 62 | 41-9 |
| 6 | 5 | 5 | 120 | 75-183 | 5 | 5 | 49 | 39-6 |
| 7 | 6 | 6 | 84 | 68-122 | 6 | 6 | 39 | 26-5 |
| 8 | 5 | 5 | 85 | 66-131 | 5 | 5 | 37 | 27-6 |
| 9 | 5 | 5 | 74 | 59-123 | 5 | 5 | 33 | 24-6 |
| 10 | 5 | 5 | 78 | 58-121 | 5 | 5 | 32 | 23-6 |
| 45-306 | 7 | 76 | 62 | 34-130 | 7 | 76 | 23.9 | 11-5 |
| 15-362 | 69 | 188‡ | 60 | 15-226 | 70 | 189‡ | 25.5 | 3-7 |

* Except as noted, all samples represent complete two four hour collections of milk from women who were receiving known diets.

‡ Samples were complete expressions of milk secreted four to eight hours by women who were eating self-chosen diets.

As early as 1914, Palmer and Eckles[31] extracted the yellow fat-soluble pigment in human milk and colostrum and divided it into two approximately equal portions by a phasic separation. Van Eekelen and de Haas,[32] also using a phasic method, found a somewhat higher percentage of carotene. Willstaedt and With,[33] on chromatographic adsorption, concluded that 20 to 30 per cent of the carotenoids of mature human milk are beta carotene. Truka-Tuzson[34] combined the phasic and chromatographic methods and found that of the total carotenoids, in colostrum 16 per cent and in milk 9 per cent, as carotene. Thompson, Kon and Mawson,[35] using chromatographic adsorption, reported 20 to 25 per cent beta carotene. Our preliminary work based on the chromatographic behavior of the carotenoids and absorption curves indicates that at most not more than 30 to 40 per cent is carotene. Assuming an average carotenoid concentration of 25 micrograms per hundred cubic centimeters and considering one fourth of it as beta carotene, we find that the vitamin A activity of the carotenoids would be about 10 U. S. P. units per hundred cubic centimeters (calculated on the basis that 0.6 microgram of beta carotene is equivalent to 1 U. S. P. or international unit). This is a small contribution in comparison with the 260 U. S. P. units of vitamin A per hundred cubic centimeters.

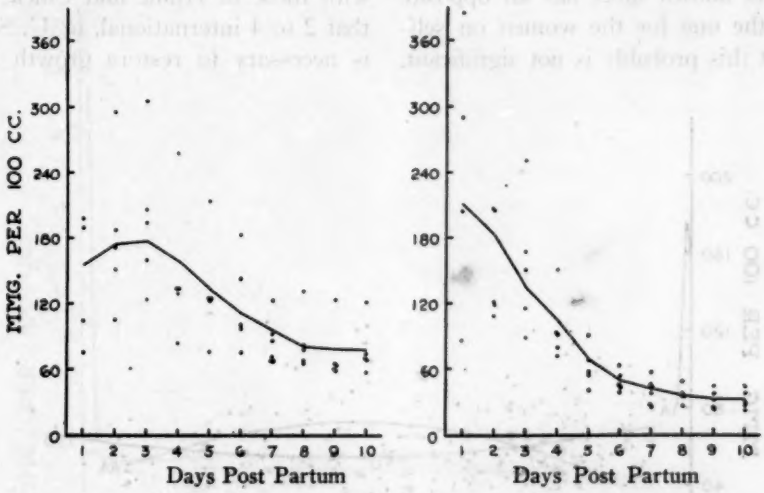



Chart 2.—Vitamin A (left) and carotenoids (right) in twenty-four hour collections of human milk from the women receiving known diets. The curves were obtained by three point smoothing of the means.

In chart 2 are scatter diagrams for the concentrations of vitamin A and carotenoids plotted against days post partum for the first ten days when the mothers were on comparable diets. Only the samples representing twenty-four hour collections are included, The smoothed means drawn on the diagram indicate rather definite trends. The concentration of vitamin A is high at the beginning, increases to a maximum on the third day and then decreases until, on the ninth and tenth days, it is approximately the average value for mature milk. The concentration on the third day is approximately three times that of mature milk, the range being from 124 micrograms (533 U. S. P. units) to 305 micrograms (1,312 U. S. P. units). The concentration of carotenoids is high on the first day and drops rapidly during the first five or six days; then it begins to level off. The concentration on the

31. Palmer, L. S., and Eckles, C. H.: Carotin, the Principal Natural Yellow Pigment of Milk Fat: Its Relations to Plant Carotin and the Carotin of the Blood Serum, Body Fat and Corpus Luteum; V. The Pigments of Human Milk Fat, J. Biol. Chem. **17**:245-249, 1914.

32. van Eekelen, M., and de Haas, J. H.: Over Carotine en vitamine A in moedermelk in het bijzonder in Colostrum, Geneesk. tijdschr. v. Nederl.-Indië **74**:1201-1208, 1934; Carotine en vitamine A in vrouwenmelk in het bijzonder in colostrum, Nederl. tijdschr. v. geneesk. **78**:2671-2673, 1934.

33. Willstaedt, H., and With, T. K.: Ueber die chromatographische Adsorptionsanalyse kleiner Carotinoidmengen (Mikrochromatographie) mit besonderer Berücksichtigung der Carotinoide der Milch und des Serums, Ztschr. f. physiol. Chem. **253**:40-46, 1938.

34. Truka-Tuzson, J.: Ueber das Lipochrom des menschlichen Kolostrum, mit besonderer Berücksichtigung des Karotins, Ztschr. f. Geburtsh. u. Gynäk. **120**: 84-90, 1939. Truka-Tuzson, J.: As Emberi Colostrum lipochromjáról Különös Tekintettel a Carotinra, Magyar orvosi arch. **41**:145-149, 1940.

35. Thompson, S. Y.; Kon, S. K., and Mawson, E. H.: The Application of Chromatography to the Study of Carotenoids of Human and Cow's Milk, Biochem. J. **36**:xvii-xviii, 1942.

tenth day, 32 micrograms per hundred cubic centimeters, is slightly above the average concentration of mature milk but is still within the average range. The average concentration on the first day is 241 micrograms per hundred cubic centimeters (range, 86 to 385 micrograms per hundred cubic centimeters), which is almost ten times that for mature milk as determined in this study.

A scatter diagram of the concentration of vitamin A during mature lactation is presented in chart 3. There does not seem to be any definite trend in the concentration from the tenth day to the three hundredth day. The curve for the group of women on the known diets has an opposite inflection from the one for the women on self-chosen diets, but this probably is not significant,

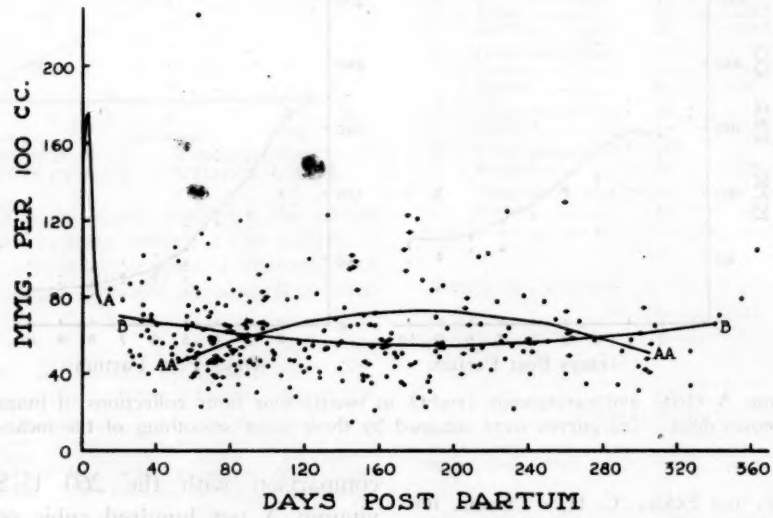



Chart 3.—Vitamin A content of mature human milk. The values for the content in twenty-four hour collection of milk from the women receiving known diets are shown with bisected points, for which the curve *AA* was obtained by the method of least squares. Curve *A* is repeated from chart 2. The points showing the values in the samples from the women eating self-chosen diets are represented by curve *B*.

and the concentration throughout this period may be considered essentially constant.

Chart 4 gives the corresponding scatter diagram and curves for the concentration of carotenoids. The curves are more comparable than those for vitamin A. Both curves, especially the one for the self-chosen diet group, show a slight tendency for the concentration to decrease as lactation progresses. It is questionable whether such a slight decrease is significant.

In earlier investigations of vitamin A in human milk, biologic methods were used. Kennedy, Palmer and Schlutz [1] reported normal or above normal growth in rats fed daily 10 cc. of individual breast milks from 4 women. Later, 10 cc. of milk was found to furnish much more than the minimum requirement of vitamin A; so the data indicate only that the concentration in the milk was not below the normal minimum. Macy and co-workers [2] found 2 cc. of human milk (pooled samples) given daily to be sufficient for normal growth of rats and 2.5 to 3 cc. daily to be necessary for the cure of xerophthalmia. Accepting the amounts given by Jung [36] (3 to international, or U. S. P., units daily) as sufficient for the cure of xerophthalmia in rats, the concentration of vitamin A in human milk, from Macy's results, would be about 100 to 2 U. S. P. units per hundred cubic centimeters. These amounts of Jung are in good agreement with those of Hume and Chick,[37] who reported that 2 to 4 international, or U. S. P., units daily is necessary to restore growth and that somewhat larger doses are necessary for the cure of xerophthalmia. Debré and Busson,[6] from experiments on rats, reported a weight gain of 8 Gm. in twenty days on 4.5 cc. of milk daily and 12 Gm. on 6 cc. daily. These data indicate a rather low concentration of vitamin A in human milk, and

36. Jung, A.: Richtlinien zur praktischen Bewertung von Vitaminhaltigen Lebens- und Heilmitteln auf Grund des Bedarfs an Vitaminen, Ztschr. f. Vitaminforsch. **1**:105-115 (April) 1932. Vogt, E.: Untersuchungen über den Vittamingehalt der Muttermilch, Zentralbl. f. Gynäk. **54**:1042-1044 (April 26) 1930.

37. Hume, E. M., and Chick, H.: Reports on Biological Standards: IV. The Standardization and Estimation of Vitamin A, Medical Research Council, Special Report Series, no. 202, London, His Majesty's Stationery Office, 1935, p. 54.

is may be explained by the fact that the subcts did not represent normal lactating women. vensson,[38] using the rat-curative method, instigated pooled samples of colostrum (milk btained between the first and second week post rtum) and found a concentration of not less an 300 and not more than 1,000 international, U. S. P., units per hundred cubic centimeters. the experiments with the mature milk from woman he determined that the vitamin A contration was between 200 and 500 international nits per hundred cubic centimeters.

Most of the recent data have been obtained the chemical method, in which the antimony

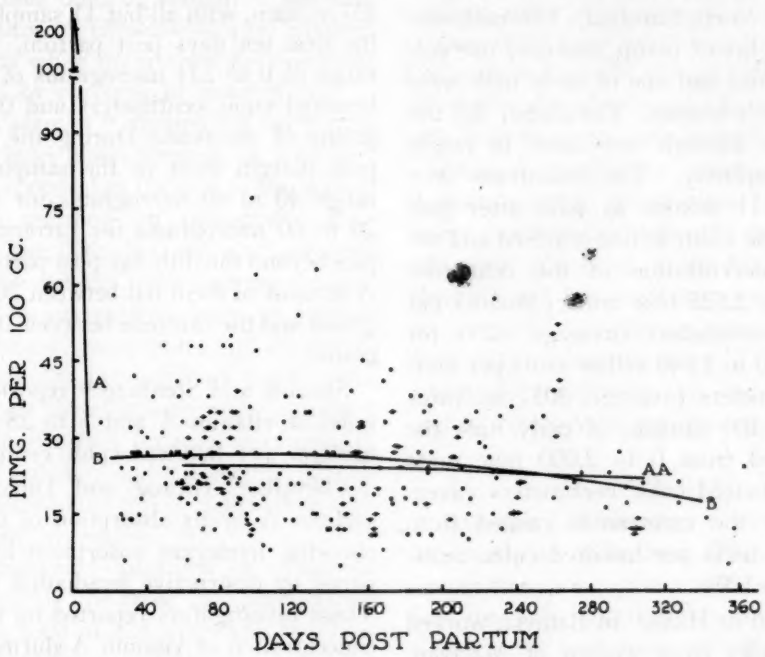



Chart 4.—Carotenoids content of mature human milk. The values for the content in twenty-four hour collections of milk from the women receiving known diets are shown with bisected points, for which the curve *AA* was obtained by the method of least squares. Curve *A* is repeated from chart 2. The points showing the values for samples from the women eating self-chosen diets are represented by curve *B*.

richloride color reaction is used. Unfortunately, uch confusion has existed in the standardizaon of vitamin A, with the result that numerous nits have appeared in the literature, with conersion factors between some of them still being ncertain. For this reason the following values re given in the units in which they were reorted.

Menken,[39] after a study of colostrum from 49 omen, reported 0 to 140 Lovibond blue units (Wolff) (average, 66 L.B.U.) of vitamin A per hundred cubic centimeters and 7 to 160 micrograms (average, 51 micrograms) of carotenoids.

Waltner,[40] in the course of a discussion of the vitamin A content of foods, reported 90 biologic units per hundred grams of colostrum and 50 biologic units per hundred grams of milk. He gave no details as to the method of analysis, the number of samples or other factors.

Van Eekelen and de Haas [32] studied milk obtained from 4 women between the second and the fourth and the eighth and the seventeenth day post partum, making analyses almost every day. For colostrum and early milk they reported concentrations of 20 to 60 Lovibond blue units (Wolff) of vitamin A per hundred cubic centimeters and 80 to 200 micrograms of carotene per hundred cubic centimeters. In later milk from 3 other women they found 5 to 10 Lovibond blue units (Wolff) of vitamin A and 10 to 20 micrograms of carotene per hundred cubic centimeters.

38. Svensson, E.: Biologische Bestimmungen des Gehaltes an A-Vitamin plus seinen Provitamin in Milch von Frauen nordischen Rasse, samt in Hagebutten und schwarzen Johannisbeeren, Skandinav. Arch. f. Physiol. **73**:237-254, 1936.

39. Menken, J. G.: Over het gehalte aan vitamine-A en carotenoiden in het bloedserum van den mensch en in moedermelk, Maandschr. v. kindergeneesk. **4**:22-35, 1934.

40. Waltner, K.: Ueber den A-Vitamingehalt einiger Nahrungsstoffe, Ztschr. f. Vitaminforsch. **3**:245-247, 1934.

Neuweiler [20b] studied milk from 69 healthy women, obtaining several samples from some of them. The 35 to 40 cc. of milk expressed for each sample was the first milk obtained from the breast in the morning. Only the earlier period of lactation was included (third to ninety-fifth day post partum). The range of concentrations for samples from women on unsupplemented diets was 25 to 300 rat units per hundred cubic centimeters for vitamin A and 0 to 400 micrograms per hundred cubic centimeters for carotenoids.[41]

Dann [42] analyzed samples of colostrum and of early milk (the latter taken just before the women left the hospital) from women of the low income group in North Carolina. The milk was expressed with a breast pump, and only one collection of colostrum and one of early milk were obtained from each woman. The author did not state whether an attempt was made to empty the breasts completely. The colostrum was obtained from 111 women as soon after parturition as possible (four to one hundred and ten hours). The concentration of the colostrum ranged from 0 to 2,625 blue units (Moore) per hundred cubic centimeters (average, 629) for vitamin A and 20 to 1,540 yellow units per hundred cubic centimeters (average, 305) for carotenoids. In the 104 samples of early milk the vitamin A varied from 0 to 2,000 blue units (Moore) per hundred cubic centimeters (average, 410) while the carotenoids ranged from 0 to 800 yellow units per hundred cubic centimeters (average, 120).

Meulemans and de Haas,[43] in Batavia, worked with 1,000 samples from women of Malayan, Chinese and European extraction. The samples were not taken in any particular manner and did not represent complete collections. The concentrations of vitamin A in colostrum (first ten days) for the three groups of subjects were: 5.4 to 80.2 (average, 26), 5.3 to 72.0 (average, 29) and 4.7 to 101.0 (average, 36) Lovibond blue units (Wolff) per hundred cubic centimeters. The concentrations in the mature milk were: 1.7 to 129.5 (average, 9), 2.6 to 50.5 (average, 10) and 5.9 to 63.5 (average, between 2 and 30) Lovibond blue units (Wolff) per hundred cubic centimeters. Carotenoids were present in the colostrum in concentrations of 6.0 to 205.5 (average, 44), 5.5 to 210.4 (average 40) and 16.0 to 362.0 (average, 67) micrograms per hundred cubic centimeters and in mature milk in concentrations of 6.0 to 45.8 (average 15), 6.2 to 62.4 (average, 14) and 8.2 to 49 (average, between 24 and 28) micrograms per hundred cubic centimeters.

Nylund [44] analyzed 583 samples of milk from 357 women, with all but 11 samples representing the first ten days post partum. He reported a range of 0 to 234 micrograms of vitamin A per hundred cubic centimeters and 0 to 422 micrograms of carotene. During the first five days post partum most of the samples were in the range 40 to 80 micrograms for vitamin A and 20 to 60 micrograms for carotene. As to samples beyond the fifth day post partum, the vitamin A in most of them fell between 20 and 60 micrograms and the carotene between 10 and 40 micrograms.

Skurnik and Stenberg [45] reported 22 to 84 rat units of vitamin A and 5 to 25 micrograms of carotene per hundred cubic centimeters.

Chevallier, Giraud and Dinard [46] measured vitamin A by its absorption of ultraviolet rays, checking irrelevant absorption by a blank prepared by destructive irradiation of the vitamin. These investigators reported on the variation in concentration of vitamin A during the course of an expression, but instead of collecting all of the milk they emphasized the importance of taking only the middle portion. They found that from the fourth to the eighth day post partum the vitamin A concentrations in the milk of 1 woman were 50, 48, 31, 31 and 43 micrograms per hundred cubic centimeters. The concentrations in samples of colostrum from 2 other women were 62 and 87 micrograms per hundred cubic centimeters. Of mature milk they analyzed 60 samples from 18 healthy women on the same regulated diet and reported a range of 6 to 47 micrograms

41. Neuweiler uses the term "carotene" since "other lipochromes do not occur in measurable quantity in milk." He does not say how he arrived at this conclusion, but a consideration of his analytic technic shows that his results are comparable to those for the carotenoids of the present study.

42. Dann, W. J.: The Transmission of Vitamin A from Parents to Young in Mammals: V. The Vitamin A and Carotenoid Contents of Human Colostrum and Milk, Biochem. J. **30**:1644-1651, 1936.

43. Meulemans, O., and de Haas, J. H.: The Carotene and Vitamin A Contents of Mother's Milk at Batavia, Indian J. Pediat. **3**:57-75 (April); 133-145 (July) 1936. de Haas and Meulemans.[12a]

44. Nylund, C. E.: Vitamin A och karotin i moders-mjölken, Finska läk.-sällsk. handl. **80**:733-766 (Sept.) 1937.

45. Skurnik, L., and Stenberg, M.: The Vitamin A and Carotene Content of Milk and Blood, Skandinav. Arch. f. Physiol. **77**:77, 1937.

46. Chevallier, A.; Giraud, P., and Dinard, C.: Sur les teneurs comparées du sang et du lait en vitamine A. Compt. rend Soc. de biol. **131**:396-398, 1939; footnote 20.

average, 18) of vitamin A per hundred cubic centimeters.[47]

Friderichsen and With[48] conducted a rather extensive study of 5 lactating women for a period of nine months. During a study period (a week a month) samples were taken every day or every second day. The breasts were emptied as completely as possible with a breast pump. The samples roughly represented twenty-four hour collections, since all of the milk produced in a day was expressed, but a constant amount and not an aliquot of each milking was used for the day's composite sample. The concentrations reported by these workers showed a range of 125 to 650 international units of vitamin A and 17.5 to 46 micrograms of carotenoids per hundred cubic centimeters while the women were on diets unsupplemented by carotene or vitamin A.

Portes and Varangot[49] studied the early milk of 7 women whose blood vitamin A was low. Four samples expressed at the end of the second feeding in the morning on consecutive days beginning with the fifth day post partum were analyzed for each subject. The average concentrations were 174 international units of vitamin A per hundred cubic centimeters and 78 micrograms of carotenoids per hundred cubic centimeters. Although these investigators expressed the belief that their values for milk were normal, the selection of subjects and the method of collecting the samples make direct comparison with the present study impossible.

Escudero[50] analyzed mixtures of colostrum samples representing the same day post partum obtained from a total of 228 women in two hospitals. The concentrations of vitamin A for the second, third and fourth days were reported as 170.3, 218.9 and 218.9 international units per hundred grams, respectively, for one group of women and for the second, third, fourth and fifth days, 316, 99.9, 256 and 108 international units per hundred grams, respectively, for the other group. The corresponding concentrations of "provitamin A" were 316.9, 208.6 and 106.6 international units for the second, third and fourth days for the first group and 165.8, 126.9, 82.3 and 134.3 international units for the second, third, fourth and fifth days, for the second group.

Hrubetz, Deuel and Hanley,[51] in the course of a recent study of human milk, analyzed 40 samples of milk obtained from the second to the tenth day post partum and 45 samples of more mature milk from women on an unsupplemented diet. They found average concentrations of 331 international units of vitamin A and 56 micrograms of carotenoids per hundred cubic centimeters in the samples from the second to the tenth day, and 206 international units of vitamin A and 39 micrograms of carotenoids in mature milk.[52]

Truka-Tuzson,[34] by combined phasic and chromatographic separations, determined the actual carotene content of 3 pooled samples of colostrum (obtained on the first three days post partum) and 1 pooled sample of milk (obtained on the eighth day post partum). The concentrations in the colostrum were 13, 24 and 26 micrograms per hundred grams and that in the milk was 3 micrograms per hundred grams.

## SUMMARY

Analyses for vitamin A were made on 314 collections of milk from 76 women: 50 were twenty-four hour collections obtained during the first ten days post partum from 6 women; 76 were twenty-four hour collections obtained between two and ten months after delivery from 7 women; 189 were samples representing four to eight hours' secretion of milk obtained within one year after parturition from 70 women. The twenty-four hour collections were procured from mothers who were receiving known diets.

The average concentration of vitamin A in mature milk was 62 micrograms (267 U. S. P. units) per hundred cubic centimeters for the women on known diets and 60 micrograms (258 U. S. P. units) per hundred cubic centimeters for the women on self-chosen diets. The corre-

47. The fact that these concentrations are lower than those reported in the present paper may be partially accounted for by the use of only the middle portion of the milk obtained at one expression and the consequent disregard for the large amount of vitamin A in the strippings.

48. Friderichsen, C., and With, T. K.: Ueber den Gehalt der Frauenmilch an Karotinoiden und A-Vitamin, besonders in Bezug auf seine Abhängigkeit von der Kost, Ann. pædiat. **153**:113-143, 1939.

49. Portes, L., and Varangot, J.: Sur la richesse actuelle du lait de femme en caroténoides et en vitamine A au début de la lactation, Compt. rend. Soc. de biol. **136**:168-170, 1942.

50. Escudero, P.: La función biológica del calostro en la alimentación del recién nacido, Resenha clin.-cient. **12**:409-413 (Nov. 1) 1943.

51. Hrubetz, M. C.; Deuel, H. J., and Hanley, B. J.: Studies on Carotenoid Metabolism: V. The Effect of a High Vitamin A Intake on the Composition of Human Milk, J. Nutrition **29**:245-254 (April) 1945.

52. The authors report their results as carotene, but the consideration of analytic procedure discloses that their "carotene" is comparable to the "carotenoids" of this paper.

sponding concentrations of carotenoids were 24 and 25 micrograms per hundred cubic centimeters for the respective groups.

The average concentration of vitamin A was high on the first day of lactation (142 micrograms or 611 U. S. P. units per hundred cubic centimeters), rose to a maximum on the third day (198 micrograms or 851 U. S. P. units per hundred cubic centimeters) and then decreased to approximately the average concentration in mature milk on the ninth and tenth days. The average concentration on the third day was approximately three times that in mature milk.

The concentration of carotenoids on the fir day of lactation (241 micrograms per hundr cubic centimeters) was about ten times that i mature milk (25 micrograms per hundred cub centimeters). It decreased rapidly until the fift and sixth days, when it began to level off. O the tenth day the concentration (32 microgram per hundred cubic centimeters) was still slightl above the average for mature milk. Both vita min A and carotenoids tended to maintain th same concentrations throughout the period o lactation from the thirtieth to the three-hundredt day after parturition.

# HUMAN MILK STUDIES

## XXVII. COMPARATIVE VALUES OF BOVINE AND HUMAN MILKS IN INFANT FEEDING

JOHN M. LAWRENCE, PH.D.; B. L. HERRINGTON, PH.D., AND L. A. MAYNARD, PH.D.
ITHACA, N. Y.

The food of many very young infants is cow's milk, although there is wide agreement among pediatricians that human milk is preferable. However, since the latter often is not supplied, it would be highly desirable to be able to modify cow's milk to provide a formula more nearly comparable to human milk in composition. The papers of this series present a comprehensive picture of the vitamin contents of human and bovine milks. Any approach to the problem of modifying cow's milk so that the formula will be a more adequate substitute for the food Nature intended must be based on a thorough knowledge of the composition of these two sources of infant nourishment.

In comparing the milks secreted by the cow and the human being, certain species differences should be borne in mind. The cow has been bred for many generations with the objective of obtaining animals which will produce large amounts of milk during a comparatively long lactation period. The quantity of colostrum secreted by the cow is much greater than the amount provided by the human being. Cow's milk is not considered normal and marketable until five days after parturition. The food consumed by the cow has little variety, the most important change being a seasonal one due to the cow's being on or off pasture. The nature of the cow's food may have little effect on the production of certain milk constituents, for the cow is a ruminant with extensive flora and fauna in her digestive tract which can consume or produce these constituents. Finally, it is noteworthy that the milk obtained by the baby from his mother has gone through none of the commercial handling which cow's milk undergoes before it is fed to infants.

This paper gives a summary of the most recent studies of the constituents of cow's milk and the factors affecting their concentrations in market milk and compares cow's milk with woman's milk as a basis for infant nutrition.

From the School of Nutrition and Department of Dairy Industry, Cornell University.

### VITAMIN A AND CAROTENE

The vitamin A and carotene contents of cow's milk are variable, and consequently it is difficult to give a satisfactory value for typical vitamin A potency. The greatest variation in market milk is caused by seasonal changes in the cows' diet. The average of mean values given by twelve different investigators since 1933 yields values of 33 micrograms of vitamin A and 30 micrograms of carotene per hundred cubic centimeters (table). If 0.25 microgram of vitamin A and 0.6 microgram of carotene each is considered as equivalent to 1 U. S. P. or international unit of vitamin A activity,[1] the average potency for milk would be 182 U. S. P. or international units per hundred cubic centimeters. These figures are not unreasonable. For midsummer milk they might be 50 per cent higher and for midwinter milk perhaps 50 per cent lower. The seasonal variation in carotene appears to be somewhat greater than that in vitamin A.

Normally the carotene present in cow's milk is almost entirely the biologically more active beta isomer.[2] Xanthophylls also are present in small amounts among the fat-soluble pigments.[3] Other pigments may be present under certain conditions.[4] In contrast, not more than 30 to

1. Berl, S., and Peterson, W. H.: Determination and Content of Carotene and Vitamin A in Wisconsin Butter, J. Nutrition **26**:527-538 (Nov.) 1943.

2. Strain, H. H.: Carotene: IX. Carotenes from Different Sources and Some Properties of α and β Carotene, J. Biol. Chem. **111**:85-93 (Sept.) 1935. Gillam, A. E., and el Ridi, M. S.: The Carotene of Milk Fat (Butter), Biochem. J. **31**:251-253 (Feb.) 1937.

3. Gillam, A. E.: A Modified Spectrophotometric Method for the Assay of Carotene and Vitamin A in Butter, Biochem. J. **28**:79-83, 1934. de Haas, J. H., and Meulemans, O.: The Carotene and Vitamin A Content of Cow Milk and Its Products, Geneesk. tijdschr. v. Nederl.-Indië **77**:279-288 (Feb. 2) 1937; abstracted, Chem. Abstr. **31**:3579, 1937.

4. Johnson, B. C.; Peterson, W. H., and Steenbock, H.: Butterfat and Silage Carotenoids, J. Dairy Sc. **24**:813, 1941.

40 per cent of the total carotenoids in mature human milk is carotene.[5]

The vitamin A and the carotene content of milk are not reduced by pasteurization,[6] commercial sterilization,[7] or irradiation with ultraviolet rays to produce 400 U. S. P. units of vitamin D per quart.[8] Vitamin A and carotene are stable for at least four months in frozen milk[9] and for at least eight months in storage in butter.[1] No losses occur in the preparation of evaporated or dried milk or in the storage of these processed milks in a cool, dry place for as long as a year.[10]

### VITAMIN D

As with vitamin A, it is rather misleading to speak of a normal vitamin D content for milk. The exposure of the cow to sunlight appears to be the most important factor in variation. However, from a consideration of the published data, it seems possible to say that normal summer milk may contain from about 2 to 4 U. S. P. units (1 U. S. P. unit equals 1 international unit) per hundred cubic centimeters and winter milk perhaps one-fourth of this (table). Weckel[11] stated that the vitamin D in normal milk is present mostly as $D_3$ (activated 7-dehydrocholesterol) and $D_2$ (activated ergosterol). It is apparent that milk is not normally an important source of vitamin D, but "vitamin D milk" has been produced by incorporating the vitamin in the milk,[12] by feeding the cows irradiated yeast[13] or by irradiating the milk with ultraviolet rays.[14] Weckel[11] stated that vitamin D is quite stable in milk.

Clinical experience demonstrates that under present living conditions neither human milk nor cow's milk can always be relied on to protect babies against rickets, although rickets is universally less frequent among nursing infants than among bottle-fed babies. Human milk may contain from 0.4 to 10 U. S. P. units of vitamin D per hundred cubic centimeters.[15] Even when vitamin D is given to the mother or when she herself is irradiated, her milk cannot be relied on to protect the infant from rickets, for vitamin D is not excreted in the milk in adequate amounts.[16]

*Vitamin Contents of Fresh Mature Human and Bovine Milk*

| Vitamin, Concentration per 100 Cc. | Human Milk * | Cow's Milk |
|---|---|---|
| Vitamin A, micrograms | 65 | 33 |
| Carotene, micrograms † | 25 | 30 |
| Vitamin D, U. S. P. units | 0.4 to 10 | 0.5 to 4.0 |
| Ascorbic acid, milligrams | 5 | 2‡ |
| Thiamine, micrograms | 14 | 38 |
| Riboflavin, micrograms | 37 | 200‡ |
| Nicotinic acid, micrograms | 183 | 85 |
| Pantothenic acid, micrograms | 246 | 350 |
| Pyridoxin, micrograms | 4§ | 67 |
| Biotin, micrograms | 0.8 | 3 |
| Inositol, milligrams | 33§ | 18 |
| Folic acid, micrograms | 45§ | 5 |

* The data were collected by the Children's Fund of Michigan except as noted.

† In human milk not more than 30 to 40 per cent of the carotenoid substances is carotene, in contrast to cow's milk, in which nearly all of the carotenoid substances is beta carotene.

‡ Very large losses of ascorbic acid and riboflavin may occur during the processing and delivery of cow's milk. Small losses of thiamine may also occur. The other vitamins, in most cases are known to be stable. (See text).

§ This value is drawn from Williams, Cheldelin and Mitchell.[5]

### ASCORBIC ACID

When the proper commercial processing practices are used, cow's milk may be an important

5. Lesher, M.; Brody, J. K.; Williams, H. H., and Macy, I. G.: Human Milk Studies: XXVI. Vitamin A and Carotenoid Contents of Colostrum and Mature Human Milk, Am. J. Dis. Child., this issue, p. 182.

6. Elvehjem, C. A.: No Objection to Pasteurized Milk on a Nutritional Basis, Milk Plant Monthly **30**: 26 (July) 1941.

7. Weckel, K. G.: Milk Should Be Thought of More as a Maintenance Food Rather Than as a Special Dietary Food, Milk Plant Monthly **27**:43 (Aug.) 1938.

8. Fuhr, I.; Dornbush, A. C., and Peterson, W. H.: The Effect of Ultra-Violet Irradiation on the Vitamin A, Carotene and Riboflavin Content of Milk, J. Dairy Sc. **26**:643, 1943.

9. Grayson, R. V.: Freezing Fresh Milk: Is This Method of Preserving a Solution to the Surplus Problem? Milk Dealer **24**:30 (April) 1935.

10. Henry, K. M.; Houston, J.; Kon, S. K., and Osborne, L. W.: The Effect of Commercial Drying and Evaporation on the Nutritive Properties of Milk, J. Dairy Research **10**:272, 1939.

11. Weckel, K. G.: Vitamin D in Milk—A Review, J. Dairy Sc. **24**:445, 1941.

12. Post, C. I.: How to Add Vitamin D Concentrate and Control Vitamin D in Milk, Food Industry **13**:41 (June) 1941.

13. Frey, C. N.: How to Get Vitamin D into Milk by Feeding Cows Yeast, Food Industry **13**:46 (June) 1941.

14. Weckel, K. G.: Irradiation and Control of Vitamin D in Milk, Food Industry **13**:43 (June) 1941.

15. Hess, A. F., and Weinstock, M.: A Study of the Antirachitic Factor in Human and in Cow's Milk, Am. J. Dis. Child. **34**:845-853 (Nov.) 1927. Outhouse, J.; Macy, I. G., and Brekke, V.: Human Milk Studies: V. A Quantitative Comparison of the Antirachitic Factor in Human and Cow's Milk, J. Biol. Chem. **78**:129-14 (June) 1928.

16. Hess, A. F., and Weinstock, M.: Rickets as Influenced by the Diet of the Mother During Pregnancy and Lactation, J. A. M. A. **83**:1558-1562 (Nov. 15) 1924. Hess, A. F.; Weinstock, M., and Sherman, E. Antirachitic Properties Developed in Human Milk by Irradiating the Mother, ibid. **88**:24-26 (Jan. 1) 192 Barnes, D. J.; Cope, F.; Hunscher, H. A., and Macy, I. G.: Human Milk Studies: XVI. Vitamin D Potency as Influenced by Supplementing the Diet of the Mother During Pregnancy and Lactation with Cow's Milk Fortified with a Concentrate of Cod Liver Oil (A Test on Rachitic Infants and Rats), J. Nutrition **8**:647-65 (Dec.) 1934.

ource of ascorbic acid. Most investigators have eported between 2.0 and 2.5 mg. of ascorbic cid per hundred cubic centimeters of milk as it s obtained from the cow (table). Unfortuately, the milk which the consumer receives sually contains only a small percentage of this, hough the amounts vary widely, depending on nethods of processing and periods of storage. The ascorbic acid in milk as it leaves the cow s all in the reduced form,[17] but as it undergoes arious processes on the way to the consumer, he ratio of reduced to dehydro (reversibly xidized) ascorbic acid varies, depending on the reatment. Both forms are physiologically ctive.

Losses of vitamin C between the time the nilk leaves the cow and the time it is used by he ultimate consumer are much more impor- ant than variations in the fresh milk. Much f the market milk as used contains practically o ascorbic acid.[18]

Various studies have shown that market pas- eurized milk is decidedly lower in vitamin C han is raw milk. Hand[17b] appears to have ssessed correctly the relative importance of the arious factors involved. Generally, exposure f the milk to light is the most important cause f loss. Between the time the milk leaves the ow and the time it is pasteurized, there occurs oth the formation and the destruction of de- ydroascorbic acid, the rate of the former process eing the limiting factor. The rate of oxidation s directly related to the concentration of oxygen resent, the degree of exposure to light and the mount of copper present. At this stage the de- rease in total ascorbic acid may not have been reat, but there will usually have been a consider- ble increase in the dehydro form at the expense f the reduced form. During pasteurization, all of he dehydroascorbic acid, whether it was orig- nally present or was formed during pasteuriza- ion, is destroyed. Immediately after pasteuriza- ion, the ascorbic acid is again all in the reduced orm, but there is ordinarily considerable oxygen resent,[18] and this leads to the formation of lehydroascorbic acid, especially when there is xposure to light and dissolved copper. This xidized form is relatively unstable and is soon ost. It is possible, however, with care in avoid- ing exposure to light and contamination with copper and by the use of short time–high temperature pasteurization, especially when this is accompanied by a deaeration process,[18] to produce market milk in which nearly all of the original vitamin C has been retained.

As would be expected, the ascorbic acid contents of powdered and evaporated milks are variable, and considerable loss is indicated in these as compared with fresh milk.[19] The vitamin C in evaporated milk gradually decreases in storage.[10] Irradiated "vitamin D milk" is said to have little ascorbic acid.[17a]

The vitamin C content of breast milk varies with the diet of the mother,[20] but in general the vitamin is present in higher concentration than in cow's milk, the average values for mature milk being 5 mg. and 2 mg. per hundred cubic centimeters, respectively (table). Although breast milk may meet the recommended allowances for the baby, it is a matter of safety to provide additional vitamin C for both the breast-fed and the formula-fed baby.

### RIBOFLAVIN

Of the B vitamin group the most important by far in cow's milk is riboflavin. Normal market milk that has not been exposed to light contains about 200 micrograms of total riboflavin per hundred cubic centimeters, depending somewhat on the breed of cows from which the milk was obtained and perhaps on the season of the year in which the milk was produced (table).

Riboflavin is relatively stable to heat at the normal $p_H$ of milk. Sure and Ford[21] found no loss when milk was held at 37 C. for twenty-two hours. Riboflavin is apparently completely stable in frozen milk, as milk has been reported held under freezing conditions for two years with no loss.[9] The most serious cause of loss of riboflavin in market milk is exposure to light. A few hours' exposure in clear bottles to direct sunlight causes loss of as much as half the ribo-

17. (*a*) Woessner, W. W.; Elvehjem, C. A., and Schutte, H. A.: The Determination of Ascorbic Acid in Commercial Milks, J. Nutrition **18**:619-626 (Dec.) 1939. (*b*) Hand, D. B.: Reduced and Total Vitamin C in Milk, J. Dairy Sc. **26**:7, 1943.

18. Sharp, P. F.; Guthrie, E. S., and Hand, D. B., in Proceeding of Laboratory Section, Thirty-Third Annual Convention, International Association of Milk Dealers, Oct. 21, 1940.

19. Woessner, W. W.; Elvehjem, C. A., and Schutte, H. A.: The Determination of Ascorbic Acid in Evaporated Milk, Powdered Milk and Powdered Milk Products, J. Nutrition **20**:327-338 (Oct.) 1940. Henry and others.[10]

20. Selleg, I., and King, C. G.: The Vitamin C Content of Human Milk and Its Variation with Diet, J. Nutrition **11**:599-606 (June) 1936. Munks, B.; Robinson, A.; Williams, H. H., and Macy, I. G.: Human Milk Studies: XXV. Ascorbic Acid and Dehydroascorbic Acid in Colostrum and Mature Human Milk, Am. J. Dis. Child., this issue, p. 176.

21. Sure, B., and Ford, Z. W.: Influence of Incubation at 37° C. on Stability of Thiamine and Riboflavin in Cow's Milk, Proc. Soc. Exper. Biol. & Med. **54**: 83-85 (Oct.) 1943.

flavin.[22] It has been reported that milk suffers little [23] or no loss [8] during irradiation for vitamin D enrichment.

In line with the stability of the riboflavin in milk to heat, all reports [24] but one [23] agree that there is no loss in pasteurization of milk, and that one attributed the loss to exposure to light during the process. Riboflavin appears to be stable during sterilization [25] and during the preparation of evaporated,[26] dried [27] and homogenized [24e] milks.

Breast milk [28] contains much less riboflavin than cow's milk, 37 micrograms, compared with 200 micrograms, per hundred cubic centimeters (table).

### THIAMINE

Less attention has been given to the thiamine than to some of the other vitamins in cow's milk. An average of the figures given in some fourteen published studies provides a value for the thiamine content of normal cow's milk, namely, 35 to 40 micrograms of total thiamine per hundred cubic centimeters. Some uncertainties arise from the limitations of present methods of determining thiamine and from the fact that thiamine appears to be present as free thiamine, as thiamine pyrophosphate (cocarboxylase) and as thiamine or cocarboxylase bound presumably to protein. There is some disagreement as to which of these forms are present, but this arises from differences in interpretation of results, and perhaps from differences in the type of milk studied. Halliday and Deuel [29] found about 40 per cent of the total thiamine to be nondialyzable until after enzymic treatment and considered this 40 per cent to be thiamine bound to protein. Houston, Kon and Thompson [30] found a varying proportion to be not ultrafilterable, but they looked on this as due to cocarboxylase bound to protein. They also found that the difference between free and total thiamine decreased as lactation progressed, becoming slight in mid-lactation. This rise in the concentration of free thiamine was accompanied by a rise in the concentration of phosphatase; so it was concluded that by midlactation the phosphatase had hydrolyzed all the cocarboxylase to free thiamine by the time the determination was made. DeJong [31] supported this conclusion. Neuweiler [32] reported 30 per cent of the thiamine in the phosphorylated form and may have been dealing with early lactation milk.

Thiamine is noted as one of the vitamins most unstable toward heat in neutral or alkaline solutions.[33] There is general agreement that part of the thiamine is destroyed in the pasteurization of milk. The short time–high temperature process probably causes the least loss, and a loss of about 3 per cent has been reported[24d]

22. (*a*) Peterson, W. J.; Haig, F. M., and Shaw, A. O.: Destruction of Riboflavin in Milk by Sunlight, J. Am. Chem. Soc. **66**:662, 1944. (*b*) Stamberg, O. E., and Theophilus, D. R.: Photolysis of Riboflavin in Milk, J. Dairy Sc. **28**:269, 1945. (*c*) Ziegler, J. A.: Photochemical Destruction of Vitamin $B_2$ in Milk, J. Am. Chem. Soc. **66**:1039, 1944.

23. Ziegler, J. A., and Keevil, N. B.: Photochemical Destruction of Riboflavin in Milk and Losses During Processing, J. Biol. Chem. **155**:605-606 (Oct.) 1944.

24. (*a*) Houston, J.; Kon, S. K., and Thompson, S, Y.: The Effect of Commercial Pasteurization and Sterilization on the Vitamin $B_1$ and Riboflavin Content of Milk as Measured by Chemical Methods, J. Dairy Research **11**:67, 1940. (*b*) Holmes, A. D.; Jones, C. P.; Wertz, A. W., and Kuzmeski, J. W.: The Ratio of Ascorbic Acid, Riboflavin and Thiamine in Raw and Pasteurized Milk, J. Nutrition **26**:337-345 (Oct.) 1943. (*c*) Holmes, A. D.: Effect of Pasteurization on the Riboflavin Content of Milk, J. Am. Dietet. A. **20**:226-227 (April) 1944. (*d*) Holmes, A. D.; Lindquist, H. G.; Jones, C. P., and Wertz, A. W.: Effect of High-Temperature-Short-Time Pasteurization on the Ascorbic Acid, Riboflavin and Thiamine Content of Milk, J. Dairy Sc. **28**:29, 1945. (*e*) Theophilus, D. R., and Stamberg, O. E.: The Influence of Breed, Feed, and Processing on the Riboflavin Content of Milk, ibid. **28**:259, 1945.

25. Wallis, G. C., and Olsen, T. M.: Effect of Season and Feeds on the Vitamin D Content of Milk Under South Dakota Conditions, Bulletin 321, South Dakota Agricultural Experiment Station, 1938, p. 3. Houston and others.[24a]

26. (*a*) Sharp, P. F., and Hand, D. B.: Riboflavin, Vitamin C and Flavor of Dairy Products, in Proceedings of the Food Conference, Institute of Food Technologists, June 1940, p. 139. (*b*) Daniel, L., and Norris, L. C.: Riboflavin Content of Milk and Milk Products, Food Research **9**:312-318 (July-Aug.) 1944.

27. Davis, H. J., and Norris, L. C.: The Effect of Process of Manufacture on the Vitamin G Content of Dried Skim Milk, J. Dairy Sc. **19**:1, 1936. Sharp and Hand.[26a]

28. Roderuck, C. E.; Coryell, M. N.; Williams, H. H., and Macy, I. G.: Human Milk Studies: XXIV. Free and Total Riboflavin Contents of Colostrum and Mature Human Milk, Am. J. Dis. Child., this issue, p. 171.

29. Halliday, N., and Deuel, H. J., Jr.: The Presence of Free and Combined Thiamine in Milk, J. Biol. Chem. **140**:555-561 (Aug.) 1941.

30. Houston, J.; Kon, S. K., and Thompson, S. Y.: The Vitamin $B_1$ and Riboflavin of Milk: II. The Different Forms of Vitamin $B_1$ in Milk, J. Dairy Research **11**:151, 1940.

31. DeJong, S.: Aneurine in Cow and Goat Milk, Enzymologia **10**:253, 1942; abstracted, Chem. Abstr. **37**:5503, 1943.

32. Neuweiler, W.: The Vitamin $B_1$ Content of Milk, Klin. Wchnschr. **20**:1072 (Oct. 25) 1941; abstracted, Chem. Abstr. **37**:3840, 1943.

33. Booth, R. G.: The Thermal Decomposition of Aneurin and Co-Carboxylase at Varying Hydrogen Ion Concentrations, Biochem. J. **37**:518-522 (Oct.) 1943. Beadle, B. W.; Greenwood, D. A., and Kraybill, H. R.: Stability of Thiamine to Heat: I. Effect of $p_H$ and Buffer Salts in Aqueous Solutions, J. Biol. Chem. **149**: 339-347 (Aug.) 1943.

this method. During the more common older process losses of from 10 to 25 per cent have been found,[34] with the first figure most common. From sterilization, losses of one third[7] and as much as one half[24a] have been reported. The thiamine in evaporated milk is reduced by 20 to 60 per cent[35] (based on the original whole milk). The wide spread of these figures is probably due to the fact that during storage the thiamine content of evaporated milk continuously decreases.[35a, c] Henry and coworkers[10] reported that no loss occurred in the preparation of dried milks, while Kendall[34a] reported that reconstituted dried milk has some 0 to 60 per cent less thiamine than whole milk. The thiamine content of milk is not reduced by five days' storage at 5 C.[34a] but is reduced 6 per cent in twenty-two hours at 37 C.[21]

Mature human milk contains on the average 4 micrograms of thiamine per hundred cubic centimeters[36] in contrast to 38 micrograms in cow's milk (table).

### NICOTINIC ACID (NIACIN)

Most of the reports which have appeared on the nicotinic acid content of milk samples indicate that the normal value is in the neighborhood of 80 to 90 micrograms per hundred cubic centimeters,[37] although a few of the reports give much higher values. This would suggest that milk is not a particularly rich source of nicotinic acid, for whole wheat bread has been reported[38] to contain 28 mg. per kilogram. On the surface it is difficult to reconcile this finding with the early observation[39] that milk is protective against pellagra. We[40] have investigated the possibility of the presence in milk of substances similar to those found in some materials by Krehl and Strong,[41] materials which become nicotinic acid active on treatment with strong alkali or acid. None was found. Very likely the explanation of the beneficial effect in pellagra is that in all of the cases in which milk was found to assist in alleviating pellegra, other deficiencies were concomitant, and the milk must have supplied other vitamins, perhaps riboflavin, valuable with respect to these complicating deficiencies.

Our studies[40] showed no loss of nicotinic acid from cow's milk (*a*) exposed in a milk bottle to direct sunlight for two hours, (*b*) pasteurized by the holder process or by the short time–high temperature process or (*c*) stored in the frozen state at about —14 C. for two months. Hodson[37e] found that irradiated evaporated milk and dried milks on reconstitution have as much nicotinic acid as fresh whole milk.

Breast milk is much richer in nicotinic acid[42] than cow's milk (table), containing 183 micrograms, in contrast to 85 micrograms in cow's milk per hundred cubic centimeters.

### PANTOTHENIC ACID AND BIOTIN

Few reports on which to base an estimate of a reasonably normal level of the pantothenic acid or of the biotin content of milk have ap-

34. (*a*) Kendall, N.: Thiamine Content of Various Milks, J. Pediat. **20**:65-73 (Jan.) 1942. (*b*) Elvehjem.[6] (*c*) Houston and others.[24a] (*d*) Holmes and others.[24b]

35. (*a*) Schlutz, F. W., and Knott, E. M.: Factors Affecting the Vitamin $B_1$ Content of Evaporated Milk, Proc. Soc. Exper. Biol. & Med. **40**:532-535 (April) 1939. (*b*) Daniels, A. L.: Further Evidence of Destruction of Thiamine in Evaporated Milk, Am. J. Dis. Child. **62**:127-129 (July) 1941. (*c*) Knott, E. M.: Thiamine Content of Milk in Relation to Vitamin $B_1$ Requirement of Infants, Am. J. Pub. Health **32**:1013-1017 (Sept.) 1942. (*d*) Henry and others.[10] (*e*) Kendall.[34a]

36. Roderuck, C. E.; Williams, H. H., and Macy, I. G.: Human Milk Studies: XXIII. Free and Total Thiamine Contents of Colostrum and Mature Human Milk, Am. J. Dis. Child., this issue, p. 162.

37. (*a*) McVicar, R. W., and Berryman, G. H.: Nicotinic Acid in Foods, J. Nutrition **24**:235-243 (Sept.) 1942. (*b*) Bailey, E. A.; Dann, W. J.; Satterfield, G. H., and Grinnells, C. D.: A Method for the Estimation of Nicotinic Acid in Milk, J. Dairy Sc. **24**:1047, 1941. (*c*) Snell, E. E., and Wright, L. D.: A Microbiological Method for the Determination of Nicotinic Acid, J. Biol. Chem. **139**:675-686 (June) 1941. (*d*) Holmes, A. D.; Jones, C. P.; Wertz, A. W.; Essler, K., and McKey, B. V.: The Ratio of Ascorbic Acid, Nicotinic and Pantothenic Acids, Riboflavin and Thiamine in Late Summer Milk, J. Dairy Sc. **27**:849, 1944. (*e*) Hodson, A. Z.: The Nicotinic Acid, Pantothenic Acid, Choline and Biotin Content of Fresh, Irradiated, Evaporated and Dry Milk, J. Nutrition **29**:137-142 (Feb.) 1945. (*f*) Williams, R. J.; Cheldelin, V. H., and Mitchell, H. K.: The B Vitamin Content of Milk from Animals of Different Species, in Studies on the Vitamin Content of Tissues, II, Publication 4237, University of Texas, 1942, p. 97.

38. Cheldelin, V. H., and Williams, R. J.: The B Vitamin Content of Foods, in Studies on the Vitamin Content of Tissues, II, Publication 4237, University of Texas, 1942, p. 105.

39. Goldberger, J.: The Cause and Prevention of Pellagra, Pub. Health Rep. **29**:2354-2357, 1914. Goldberger, J.; Waring, C. H., and Willets, D. G.: The Prevention of Pellagra: A Test of Diet Among Institution Inmates, ibid. **30**:3117-3131, 1915. Goldberger, J., and Tanner, W. F.: A Study of the Treatment and Prevention of Pellagra: Experiments Showing the Value of Fresh Meat and of Milk, the Therapeutic Failure of Gelatin, and the Preventive Failure of Butter and Cod Liver Oil, ibid. **39**:87-107 (Jan. 18) 1924.

40. Lawrence, J. M.; Herrington, B. L., and Maynard, L. A.: To be published.

41. Krehl, W. A., and Strong, F. M.: Studies on the Distribution, Properties and Isolation of a Naturally Occurring Precursor of Nicotinic Acid, J. Biol. Chem. **156**:1-12 (Nov.) 1944.

42. Coryell, M. N.; Harris, M. E.; Miller, S.; Williams, H. H., and Macy, I. G.: Human Milk Studies: XXII. Nicotinic Acid, Pantothenic Acid and Biotin Contents of Colostrum and Mature Human Milk, Am. J. Dis. Child., this issue, p. 150.

peared; most of those contain the result of one determination on a single sample of market milk. It is perhaps safe to give a figure of 300 to 400 micrograms of calcium pantothenate per hundred cubic centimeters as an average level for the pantothenic acid in milk. With respect to biotin, a difficulty arises from the great variability of levels between individual cows and individual samples.[43] With considerable reserve, one might suggest as a normal value for the biotin level of milk, about 3 micrograms per hundred cubic centimeters.

Both pantothenic acid and biotin were found to be stable in milk during two hours' exposure to direct sunlight and during pasteurization by either the holder process or the short time–high temperature method. Hodson [37e] found no decrease in the content of either of these vitamins in irradiated evaporated milk and dried milks when reconstituted, except, perhaps, a somewhat lower level of biotin in dried skim milk.

On the average, human milk [42] contains 246 micrograms of calcium pantothenate per hundred cubic centimeters compared with 350 micrograms in cow's milk. Similarly, cow's milk is superior to breast milk in biotin content, containing 3 micrograms per hundred cubic centimeters, compared with 0.8 microgram for breast milk (table).

### VITAMIN K

Anderson, Elvehjem and Gonce [44] have reported that dogs are able to grow and reproduce normally on a mineralized raw milk diet supplemented with cod liver oil. However, when pasteurized milk was substituted a hemorrhagic condition occurred which was thought to be due to vitamin K deficiency. The deficiency appeared still worse when evaporated milk was used. Sells, Walker and Owen [45] found that cow's milk powder could fill the vitamin K requirement of newborn infants. Assays by Dam and co-workers [46] gave values of from 0 to 4 Dam-Glavind units per cubic centimeter, with an average value of 2. On the other hand, Mueller and Wertz [47] could find no evidence of vitamin K in milk. Vitamin K has been shown [48] to be synthesized in the rumen of the cow.

Dam and co-workers [46] found that breast milk averaged 0.5 Dam-Glavind unit per cubic centimeter and that the level could be raised to 2 units per cubic centimeter by feeding vitamin K.

### PYRIDOXINE

There have been several reports on the pyridoxine content of cow's milk, giving values ranging from 50 to 110 micrograms per hundred cubic centimeters. Perhaps the most thorough study has been that of Hodson,[49] who found an average value of 67 micrograms per hundred cubic centimeters in 48 samples. He also reported that there is no significant loss of pyridoxine in the preparation of pasteurized, evaporated or dried milk.

Williams and associates [37f] have studied the pyridoxine content of milk from animals of different species. They found breast milk to be much lower than cow's milk in pyridoxine. Cow's milk was found to contain 67 micrograms per hundred cubic centimeters, in contrast to only 4 micrograms per hundred cubic centimeters in human milk (table).

### OTHER LITTLE-STUDIED FACTORS

Williams and associates [37f] reported 18 mg. of inositol per hundred cubic centimeters of cow's milk and less than 5 micrograms of folic acid per hundred cubic centimeters. However, the situation with regard to folic acid assay is still unsettled. There are suggestions [50] of considerable folic acid activity of some sort in milk. Williams and associates [37f] have reported breast milk to contain 33 mg. of inositol and 45 micrograms of folic acid per hundred cubic centimeters.

---

43. Lawrence and others.[40] Hodson.[37e]

44. Anderson, H. D.; Elvehjem, C. A., and Gonce, J. E.: A Comparison of the Nutritive Values of Raw, Pasteurized and Evaporated Milks for the Dog, J. Nutrition **20**:433-443 (Nov.) 1940.

45. Sells, R. L.; Walker, S. A., and Owen, C. A.: Vitamin K Requirement of the Newborn Infant, Proc. Soc. Exper. Biol. & Med. **47**:441-445 (June) 1941.

46. Dam, H.; Glavind, J.; Larsen, E. H., and Plum, P.: Investigations into the Cause of the Physiological Hypoprothrombinemia in New Born Children: IV. The Vitamin K Content of Woman's Milk and Cow's Milk, Acta med. Scandinav. **112**:210, 1942; abstracted, Dairy Sc. Abstr. **6**:41, 1944.

47. Mueller, W. S., and Wertz, A. W.: Vitamin K Content of Dairy and Cacao Products, J. Dairy Sc. **28**:167, 1945.

48. McElroy, L. W., and Goss, H.: A Quantitative Study of Vitamins in the Rumen Contents of Sheep and Cows Fed Vitamin-Low Diets: I. Riboflavin and Vitamin K, J. Nutrition **20**:527-540 (Dec.) 1940.

49. Hodson, A. Z.: The Pyridoxine Content of Fresh, Pasteurized, Evaporated and Dried Milk, J. Nutrition **27**:415-418 (May) 1944.

50. Welch, A. D., and Wright, L. D.: The Possible Existence of a Microbiologically Inactive "Folic Acid"-Like Material Possessing Vitamin Activity in the Rat, Science **100**:153-154 (Aug. 18) 1944.

Engel [51] found an average value of 14.7 mg. of choline per hundred cubic centimeters for 6 milk samples. His data indicate that dried milk has suffered no destruction of choline. Hodson [37e] found an average value for 10 samples of 14.7 mg. per hundred cubic centimeters and no evidence of losses in the preparation of evaporated or dried milks.

51. Engel, R. W.: The Choline Content of Animal and Plant Products, J. Nutrition **25**:441-446 (May) 1943.

## COMMENT

A subsequent paper on the comparison of the nutriments in bovine and human milks will include equally important information on the proximate composition, the distribution of minerals, amino acids, fatty acids and other essential components.

## ACKNOWLEDGMENT

The data contributed to this review by work in this laboratory was obtained in the course of a research project supported by the Nutrition Foundation, Inc., of New York.

# Book Reviews

**The Male Hormone.** By Paul De Kruif. Price, $2.50. Pp. 243, with no illustrations. New York: Harcourt, Brace and Company, Inc., 1945.

De Kruif writes the story of the male sex hormone with his usual ability to dramatize scientific observations.

Dr. H. N. Bundesen, health commissioner of Chicago, was responsible for De Kruif's personal sampling of the effects of the hormone. Dr. Bundesen also gave him new ideas about its possibilities as a "life-builder," in contradistinction to the ideas he had previously held about the irreversibility of old age, as stressed by his teacher of pathology, Dr. A. S. Warthin, of the University of Michigan.

De Kruif outlines the history of the hormone, pointing out that Brown-Séquard put it into the scientific "dog-house" from 1889 until 1926, when Koch and his associates began a new epoch of chemical investigation. By 1936, Butenandt, Laqueue and Ruzicka had performed their famous experiments, and the material first became available in quantity. Clinical observations showed its amazing effect on the sexual and somatic development of eunuchoid men. However, many physicians still thought it had slightly immoral aspects, and any effects other than against eunuchoidism were mere reerotization. There were, nevertheless, almost one thousand observations in the literature indicating that testosterone might have "life-building powers far beyond the sexual." De Kruif writes of its beneficial results in old age, in the so-called male climacteric, of its influence on muscle tone—in short, of its power to improve "total vitality." He mentions only in passing its effect on growth. He dwells at some length on its effects in insanity, obliterative arterial disease, angina pectoris and other ailments. Although a prominent Eastern observer doubts its efficacy against angina pectoris, De Kruif toys with the idea of a big program involving the administration of testosterone long enough to find out whether it would prolong life. The value of testosterone in treatment of most of these conditions is certainly open to question, although it has been proved to be effective in controlling the symptoms of the male climacteric, and much of the evidence presented by De Kruif in support of his contention is subjective.

The book provides interesting reading, but there is not a sufficiently sharp line of demarcation drawn between fact and fantasy.

**Mass Radiography of the Chest.** By H. E. Hilleboe, M.D., and R. H. Morgan, M.D. Price, $3.50. Pp. 288, with 93 figures and 11 tables. Chicago: The Year Book Publishers, Inc., 1945.

The greatest value of this book is the assistance offered to persons about to begin mass radiography of the chest. In the 288 pages, all known phases of this recently developed and accepted procedure are included. The book covers in detail preliminary planning with medical societies, local health officers, community and industrial organizations and hospitals and the selection of equipment to be used, as well as practical methods of transporting, installing and operating equipment, interpreting films and recording and filing of the data obtained. Methods are shown by which one employee may handle as many as 2,500 patients in a week and by which a factory employee may lose only fifteen minutes from work to have the examination made.

Photography of the fluoroscopic image on miniature film, the basis of mass radiography, is of special interest because of the decrease in the cost and the speed with which hundreds of examinations of the chest can be made. This photofluorography is not new. It was the object of experimentation in 1897, two years following the discovery of the roentgen ray. It first became practical in 1938 when Potter reported "highly satisfactory" results, using 4 by 5 inch (10 by 12.8 cm.) film. With the development of improved technical equipment, satisfactory results recently have been accomplished with still smaller film (70 mm. and 35 mm.). Great improvement in the quality of automatic cameras has added materially to the quality of the film. Different types of these are illustrated, with estimated prices. The greatest technical improvement, which adds accuracy and prevents retake examinations, is the "photo-timer," which stops the fluorescence of the screen when just the right exposure for proper recording has occurred. This was designed and built by one of the authors. Forty-eight pages of selected thoracic conditions are nicely illustrated and reported on.

The authors believe that after a complete survey with satisfactory miniature films, two important objectives are gained: 1. Patients with infections are isolated and prevented from spreading the disease. 2. Abnormalities are recognized early enough for effective treatment to prevent advancement of the disease and at minimal cost. The result is reduction in the number of new cases of tuberculosis, control of the disease and its eventual eradication. The reader finds few questions unanswered regarding mass radiography of the chest.